AGE-RELATED MEMORY LOSS AND NEUROBIOLOGICAL CHANGES IN THE C57BL/6 MOUSE

BY

PATRICIA JOAN KUBANIS

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1981

To Audrey Kubanis, mother and friend, for her wit, wisdom, and enthusiastic support of the endeavors of my choice.

And to Dr. Ivan Kubanis, my late father, who inspired so many through teaching the history and methodology of science.

ACKNOWLEDGMENTS

Working with the members of my supervisory committee has been a uniquely rewarding experience. I would like to thank my Chairman, Dr. Steven Zornetzer, for his insight, creativity, and optimism throughout my graduate studies. I owe a special debt of gratitude to Dr. Gerhard Freund, whose generous contributions of time, expertise, and resources made the receptor binding studies possible. The example he set, impressively combining clinical medicine and research, has been an inspiration to me. I thank Dr. Marieta Heaton for her encouragement, confidence, and friendship which I have enjoyed greatly during my graduate studies. Dr. Don Walker consistently provided me with sound advice, support, and statistical expertise. My thanks go to Dr. James Simpkins for his enthusiasm and enlightening perspective in regard to aging research. Dr. Adrian Dunn provided enlivening discussions on many subjects. I thank Dr. William Luttge, Acting Chairman of the Department of Neuroscience, for his exemplary high standards in research.

Thomas Brophy and James Scott helped me enormously in carrying out the receptor binding studies, and their presence made the lab an enjoyable and stimulating place to work. I thank Cliff Abraham for friendly interaction and

assistance with the computer. Glen Gobbel collaborated on pilot experiments for this dissertation. My thanks to Carlene Eunice for her helpfulness and efficiency at all times. Katherine Williams typed the manuscript with unusual proficiency.

I would also like to express my gratitude to Dr. Phillip Gallo, who stimulated my interest in research and Dr. Sandra Mollenauer and Dr. Rod Plotnik with whom I began my study of neuroscience at San Diego State University.

TABLE OF CONTENTS

Abstract of Dissertation Presented to the Graduate Council of the University of Florida in Partial Fulfillment of the Requirement for the Degree of Doctor of Philosophy

AGE-RELATED MEMORY LOSS AND NEUROBIOLOGICAL CHANGES IN THE C57BL/6 MOUSE

By

Patricia Joan Kubanis

August 1981

Chairman: Dr. Steven F. Zornetzer
Major Department: Neuroscience

Previous research on age-related memory loss and neurobiological alterations associated with aging has generally involved separate experiments, not necessarily using the same animal strains or species. Age-related alterations in the brain cholinergic system have been reported, and because this system has been implicated in memory function, a cholinergic hypothesis of age-related memory loss has achieved increasing popularity. This investigation was designed principally to test this hypothesis.

Memory function in mice of the C57BL/6 strain 4, 8, 15, 20, and 25 months of age was evaluated, and brain muscarinic cholinergic receptor status was determined in the same animals by regional assay of [^{3}H]quinuclidinyl benzilate (QNB) binding. Performance on passive avoidance tasks was

used to evaluate memory. The results indicated significant performance deficits in 15-, 20-, and 25-month-old mice. However, significant changes in [^{3}H]QNB binding were not observed in cerebral cortex, striatum, hippocampus, or cerebellum.

In a separate experiment, extreme age groups (4- and 29-month-old mice) were compared on regional binding of [^{3}H]QNB. Significant decreases in [^{3}H]QNB binding in the older group were observed in cerebral cortex, striatum, and hippocampus. Binding of a putative benzodiazepine receptor ligand, [^{3}H]flunitrazepam, was also significantly decreased in cerebral cortex, hippocampus, and cerebellum.

Results of an experiment intended to assess the plastic capability of the cholinergic system in aged mice were inconclusive. A pharmacological manipulation, previously shown to induce a compensatory increase in [^{3}H]QNB binding in the rat hippocampus, did not affect [^{3}H]QNB binding in mice. Therefore, the effect of age on this response could not be determined.

The results of this investigation do not support the cholinergic hypothesis of age-related memory loss. Although changes in both memory function and [^{3}H]QNB binding occurred with aging in mice, these phenomena were not temporally correlated. Memory deficits occurred at 15 months of age, while decreases in [^{3}H]QNB binding were not observed until mice were 29 months of age. Since behavioral changes

substantially preceded changes in [^{3}H]QNB binding, it appears unlikely that a simple causal relationship exists between changes in the cholinergic system and age-related memory loss.

CHAPTER I

INTRODUCTION

Background

Age-Related Memory Deficits

Human studies

The human literature on aging and long-term memory (LTM) is both voluminous and controversial. Adherents may be found to every conceivable position on this issue, including one which perceives age-related LTM deficits as cultural artifacts (e.g., Labouvie-Vief, Hoyer, Baltes, & Baltes, 1974). Nevertheless, most investigators concur that there is a genuine impairment of some aspects of LTM with aging. Much of the disagreement concerns the relative importance of acquisition versus retrieval processes in accounting for the observed deficits.

Paired-associate learning and tests of recall or recognition of word lists are the most commonly used tests of LTM capabilities of the elderly. Gilbert (1941) first reported paired-associate learning impairments in aged subjects. This finding has been replicated (see Botwinick, 1973) and is reflected in both performance deficits after a predetermined number of trials and in an increase in the number of trials to reach a criterion level of performance. Free

recall, but not recognition, of a word list was found to be impaired in older subjects by Schonfield and Robertson (1966). Other investigators have reported age-related deficits in word list recognition as well (e.g., Botwinick & Storandt, 1974; Erber, 1974, 1978). Arenberg (1978) has shown that aging impairs performance on the Benton Revised Visual Retention Test, which is a recall test of memory for designs. This result indicates that age-related recall deficits are not restricted to the verbal-memory domain. Typically, performance levels on paired-associate and recognition tasks are considered to reflect acquisition strength, while tests of recall ability are considered to reflect the adequacy of retrieval mechanisms (e.g., Crowder, 1976). It appears then that both acquisition and retrieval may be impaired with advancing age.

Pacing. Pacing, the rate of stimulus presentation and time allowed for response, is one situational variable that differentially influences the performance of older subjects on LTM tasks. Canestrari (1963) showed that older subjects benefited more than younger subjects when they were allowed to work at a slower pace or set their own pace on a paired-associate task. In a more recent study (Monge & Hultsch, 1971), increasing the inspection interval (time allowed to learn the association) improved the performance of young and old subjects equally. Increasing the anticipation interval (time allowed for response production) was selectively beneficial to older subjects, suggesting that

retrieval and/or response production take longer for the elderly.

Factors like pacing and test anxiety appear to have important effects on performance by the elderly. Several possible reasons for this phenomenon can be postulated. One possibility is that neural processing of information per se in the elderly is equivalent to that in younger adults, but that older subjects require more time to acclimate to the situation before demonstrating their true capabilities. In this case, unpaced conditions should reveal the actual memory processing abilities of older subjects. If, on the other hand, adjustment of environmental demands compensates for actual biological deficits in LTM, then making these environmental adjustments might mask a real aging deficit. The role of pacing in determining LTM recall has been and will continue to be debated, but neither of the two possibilities mentioned above can account for all of the data on aging and LTM. Even under self-paced conditions with considerable practice, age-related differences on LTM tasks persist. These deficits are discussed below.

Acquisition-encoding deficits. There is considerable evidence that some of the deficits in LTM reported under optimal conditions are due to age-related decrements in effective encoding of information. Organization of material to be remembered is one factor which facilitates retention. Hultsch (1969) found that when subjects of different ages were given a word list to learn, older subjects benefited

disproportionately when instructed to organize the list or associate each word with its first letter, rather than simply to remember the words. In other experiments reporting recall deficits, organization differences have not been found (e.g., Hultsch, 1971, Laurence, 1966). These studies were based on sorting behavior, which according to several investigators (Craik, 1977; Hultsch, 1974), may not accurately represent organization. Using a more appropriate measure of organization (from Bousfield & Bousfield, 1966), Hultsch (1974) reported that elderly subjects exhibited both recall and organization defects.

Another age-related difference in encoding concerns the use of mnemonics. Hulicka and Grossman (1967) reported that older subjects used verbal and visual mediators only half as often as young subjects on a paired-associate task. When given instructions to develop such mediators, elderly subjects improved more than their young counterparts, although the young still performed better overall. Allowing unpaced conditions did not eliminate the age differences in use of mnemonics or in recall (Hulicka, Sterns, & Grossman, 1967). This suggests that older subjects are not merely slower to develop associations. Perlmutter (1978) reported that when instructed to write down associations to words, older subjects generated the same number of associations, but tended to produce less common associations. In a further study (Perlmutter, 1979), it was reported that young subjects performed better using their own associations than using

experimenter-provided associations, while older subjects were not especially benefited by their own associations.

Summarizing the problem of encoding, we can conclude that there are age-related differences in both organization of material and in the use of mnemonics. Elderly subjects do not spontaneously use effective mediators for encoding information, but are able to utilize mnemonics if specifically instructed to do so. In addition, there appear to be qualitative differences in the relative usefulness of mediators developed.

Retrieval deficits. Many of the age-related decrements in LTM are eliminated on tests of recognition as opposed to recall. However, as previously mentioned, deficits have been reported in both situations. Recall impairments are usually greater than recognition deficits (e.g., Erber, 1974; Harwood & Naylor, 1969), but age differences in recognition are pronounced on complex tasks (Erber, 1974) and tasks including difficult distractor items (Smith, 1975). Conversely, age-related recall differences can be attenuated under cued conditions in which categories for list items are provided (Laurence, 1967). These data are all consistent with the idea that aids to retrieval can mitigate age decrements in LTM.

Craik and Masani (1969) examined retrieval capabilities with a "chunking" experiment. Subjects were presented with word lists differing in order of approximation to English, and a chunk was defined (Tulving & Patkau, 1962) as a string

of words recalled in the same order as presented. No age difference was found in the size of chunks recalled, although older subjects recalled fewer chunks. These results were interpreted to represent intact encoding (size of chunks) but impaired retrieval (number of chunks) in the elderly. A study by Buschke (1974) also implicates a retrieval disorder to account for age-related LTM deficits. Subjects were presented only once with a word list and were then given several trials for recall testing. Elderly subjects demonstrated more variability in the words they recalled on each attempt. This suggested that the words were encoded, but that older subjects were unable to retrieve them on some trials.

Acquisition vs retrieval. The acquisition-retrieval controversy will undoubtedly continue among investigators of human age-related memory dysfunction. It is probable that both processes are involved and that the cause-effect relationships are difficult to disentangle. The capability for adequate encoding of information is relatively preserved, although the elderly tend not to spontaneously utilize the most effective strategies. Providing encoding strategies or retrieval cues can dramatically improve the performance of the elderly.

The contrast between intentional and incidental learning by older subjects is relevant to this point. Craik (1977) has discussed the rationale for incidental learning paradigms and described the methodological requirements for

clear interpretation of their results. Essentially, the idea is that if older subjects are capable of efficient encoding, but ordinarily fail to use optimal strategies, then by specifically instructing subjects to perform certain operations on the material and testing them unexpectedly, acquisition should be equivalent at all ages. Differences which remain under incidental conditions may be attributed to sources other than acquisition strategy.

Johnson (1973), in one of the first of these studies, reported no age differences under incidental learning conditions in recognition, free recall, or cued recall. Under intentional conditions young subjects performed better on the free recall task. Results from several studies (Perlmutter, 1978, 1979; White, unpublished data cited in Craik, 1977) indicated that equating acquisition strategy through incidental conditions eliminated age differences in recognition, but not recall. Differences were observed in both recall and recognition after intentional learning.

Eysenck (1974) trained subjects using incidental semantic and nonsemantic tasks, and reported that recall deficits of older subjects became larger as the semantic requirements of the task increased. He suggested that older subjects were impaired in semantic processing ability. Craik (1977) and Perlmutter (1979) have questioned this interpretation, since Eysenck's study tested recall performance only, thereby confounding adequacy of encoding with retrieval capability. Both Eysenck (1974) and Perlmutter (1979)

reported that cued recall attenuated the age-related recall deficit on incidental, but not intentional items. Because incidental learning appears to eliminate recognition deficits, but not recall deficits, these data implicate differences in acquisition strategy and a residual retrieval deficit in accounting for age-associated LTM impairments.

Zelinski, Walsh, and Thompson (1978) trained young and old subjects under a variety of conditions, including intentional memorization and three incidental tasks: semantic, nonsemantic, and passive listening. Recall testing commenced 2 min or 48 hr later. Recognition was not tested. Older subjects had poorer recall at both time points. Nevertheless, at 2 min both groups demonstrated similar patterns of performance according to task, i.e., intentional or semantic incidental training resulted in better recall than did nonsemantic incidental training or passive listening. At 48 hr, however, the older subjects' performance was not influenced by different learning conditions, even though young subjects retained the same performance pattern as at 2 min. Presumably, retrieval demands are low 2 min after training and much higher 48 hr later. Zelinski et al. interpreted their findings to indicate that older subjects are capable of adequate encoding but are impaired in retrieval processes.

In conclusion, it appears that young subjects spontaneously adopt strategies for acquisition that generally improve their performance relative to older subjects. The

results of most incidental learning studies indicate that older subjects continue to demonstrate retrieval deficits (as reflected by recall testing), even when acquisition strategies are presumably identical.

Animal studies

Acquisition deficits. A number of investigators have reported the absence of age-related performance impairments on both appetitive and shock-motivated tasks. Doty (1966b) reported equivalent acquisition by young and old rats of simple shock avoidance and discriminated shock avoidance with distributed practice. Straight runway learning, 1-choice T-maze, and 4-choice T-maze problems for food reward presented no particular difficulty for old rats (Goodrick, 1972). Old and middle-aged rhesus monkeys were compared on concurrent discrimination learning in a study by Medin, O'Neil, Smeltz, and Davis (1973). Although older monkeys made more errors on the first trial of each day of training, on subsequent trials and overall the two groups performed comparably.

In other cases aging appears to have a deleterious effect on acquisition. Older rats were deficient in learning simple shock avoidance and discriminated shock avoidance if trials were massed, rather than distributed (Doty, 1966b). Several studies have indicated that shuttle box acquisition declines with aging in mice (Freund & Walker, 1971; Oliverio

& Bovet, 1966). Using a shock-motivated brightness discrimination task, Thompson and Fitzsimons (1976) found that aged rats were slower to reach criterion levels of performance than were young adult control rats.

Goodrick has done a series of studies examining the effect of aging on acquisition of 1-, 4-, and 14-choice mazes by rats. In addition to finding an age-related deficit in acquisition on the 14-choice maze (1968), and showing that the effect was related to the number of choices (1972), as previously mentioned he also reported that young animals performed better on a schedule of distributed trials, while older animals were benefited by massed trials (1968, 1973). This last finding contrasts with the adverse effect of massed trials on shock avoidance (Doty, 1966b). Old rats also consistently made more perseverative errors in these maze studies. In a further experiment, Goodrick (1975) gave rats "forced-correct-choice" training on the 14-choice maze, hypothesizing the old rats were more rigid in their behavior and would benefit from this training. The results supported this hypothesis. Performance by older rats on test trials was selectively facilited by the training when compared to younger control rats.

Bartus, Dean, and Fleming (1979) investigated visual discrimination learning in rhesus monkeys of different ages. Using a subject-paced, automated procedure they found no age deficit in acquisition of color and pattern discrimination problems to an 18/20 criterion. However, when monkeys were

subsequently trained to reverse one of their previous discriminations, aged subjects were significantly slower to extinguish the first habit and learn the reversal problem. Bartus et al. interpreted this finding as evidence for "behavioral rigidity" and/or increased susceptibility to proactive interference in older monkeys.

It may be concluded that aged animals are equivalent to young animals in acquisition of some tasks but impaired on other tasks. Task complexity, determined, for example, by the number of choice points in a maze, appears to be one factor in this distinction. A higher incidence of perseverative errors and difficulty on reversal tasks may also be characteristic of older animals' performance.

Retention deficits. Retention in aging animals has been less thoroughly studied than has acquisition ability. Relearning studies, in which young and old rats were able to relearn discriminated avoidance after 1 and 3 months (Doty, 1966a) and a multiple T-maze after 45 days (Goodrick, 1968) in a comparable number of trials, suggested that retention was not impaired by aging. However, Doty's (1968) study indicated that aged rats (421-621 days old) performed relatively poorly during relearning of discriminated avoidance 2 and 4 months after initial training.

Several more recent studies have provided evidence for age-related retention impairments. Thompson and Fitzsimons (1976) reported that relearning time on discriminated avoidance increased linearly with age. In a study using a 30-day

training-test interval, old rats (547 days old) demonstrated retention deficits on both shuttle box active avoidance and step-through passive avoidance compared to younger adult rats (McNamara, Benignus, Benignus, & Miller, 1977). However, because a passive avoidance deficit was also observed 2 min after training it is not clear whether the 30-day effect reflected acquisition problems or an actual retention deficit.

Brizzee and Ordy (1979) trained 29-month-old Fischer rats and younger rats on a passive avoidance task in which animals were shocked as they approached a food reward. Retention deficits were observed in the aged animals at 2- and 6-hr training-test intervals. Age-related step-through passive avoidance retention deficits in C57BL/6J mice have been reported at 1- and 5-day training-test intervals (Bartus, Dean, Goas, & Lippa, 1980). Deficits were first apparent in 13-month-old mice and were more pronounced in 23- and 31-month-old mice compared to young animals (2, 3, 6, or 9 months of age). Strong, Hicks, Hsu, Bartus, and Enna (1980) also reported significant performance impairments in 12- and 30-month-old C57BL/6J mice compared to 6-month-old mice on step-through passive avoidance. Using a 24-hr interval they found a 40% decrease in retention latency in the 12-month-old group and a 63% decrease in the 30-month-old group in comparison to the youngest group. The previously mentioned interpretational problem also applied to

these studies, since adequate performance shortly after training was not demonstrated in the aged animals.

Gold and colleagues have reported data on step-through passive avoidance retention of 60-day, 1-year, and 2-year-old Fischer rats using a more extensive series of training-test intervals. Young animals performed well after 1-, 7-, or 21-day intervals with retention declining at 6 weeks. One-year-old rats showed good retention at 1 day, but a performance drop at intervals of 7 days or longer. Retention in 2-year-old rats was preserved at a 2-hour training-test interval but declined within a 6-hr interval (Gold & McGaugh, 1975; Gold, McGaugh, Hankins, Rose, & Vasquez, 1981). Preliminary data reported by Gold and McGaugh on discriminated avoidance also suggested that rate of forgetting increased with advancing age.

The passive avoidance experiments by Gold and colleagues provided relatively convincing evidence of retention deficits, since aged animals performed well at short training-test intervals but performed poorly when longer intervals were examined. Data by Kubanis, Gobbel, and Zornetzer (1981) corroborate and extend these findings. These investigators compared performance of young (3-5 months) and aged (20-21 months) Swiss mice on a step-down passive avoidance task. Aged mice trained on a single-trial paradigm demonstrated performance deficits at a 24-hr training-test interval but good performance at a 2-hr interval. Nevertheless, using the single-trial paradigm an assumption is made

that strength of training is equivalent for all animals. To further dissociate acquisition and retention effects an additional experiment was performed in which animals were trained to a criterion and retention was monitored over a 10-day period. In this experiment acquisition (trials to criterion) was equivalent in the two groups, but aged mice tended to step down sooner (median = 3.5 days) than young mice (median = 10.0 days) indicating a retention deficit. These data combined with those of Gold et al. suggest that retention deficits persist in aged rodents even in the presence of adequate controls for acquisition strength.

Conclusions on age-related memory deficits

Considered together, the human and animal data provide compelling evidence for age-related memory deficits. Human research indicates that acquisition deficits are task-dependent, apparently determined by task complexity. Ineffective organizational strategies and use of mnemonics by older subjects may account for a substantial portion of reported acquisition deficits. The incidental learning studies, however, suggest that age-related memory retrieval deficits remain even when acquisition strategies have been equalized.

The animal literature generally supports these conclusions. Acquisition deficits are task-dependent, and as in humans, are related to the degree of task complexity. Retention deficits have been reported on a variety of tasks.

Several investigators have reported age-related deficits on single-trial passive avoidance tasks. Retention is difficult to evaluate in some of these studies because comparable acquisition was not demonstrated between young and aged animals. However, those experiments demonstrating good performance by aged animals shortly after training, but poor performance after longer intervals, are strongly indicative of retention deficits. These experiments, like the incidental learning paradigms in the human literature, control for acquisition strength and offer more convincing evidence of age-related memory deficits.

Cholinergic Function and Memory

The central cholinergic system has been implicated in memory function by neuropharmacological research which began in the 1960's. Deutsch and colleagues have been the principal proponents of the "cholinergic hypothesis." These investigators have shown that, in general, intracerebral administration of anticholinergic drugs (scopolamine or atropine) disrupts memory in experimental animals, while administration of anticholinesterase drugs (physostigmine or diisopropyl fluorophosphate), which potentiate the cholinergic system by inhibiting degradation of acetylcholine (ACh), results in facilitation of memory (e.g., Deutsch, 1973). These effects are dose- and time-dependent. Anticholinergic agents have also been shown to disrupt memory in humans (Cutting, 1964; Drachman & Leavitt, 1974).

Deutsch's hypothesis states essentially that cholinergic synapses are modified by learning, such that over time the postsynaptic membrane becomes more sensitive to ACh. After a certain period of time, sensitivity declines and forgetting ensues. Irrespective of the validity of this particular memory model, the data base indicating that cholinergic drugs influence memory is substantial. Evidence supporting age-related changes in cholinergic function is described below.

Cholinergic Function and Aging

Research interest in the effect of aging on cholinergic neurotransmitter systems has dramatically increased recently. Levels of ACh are difficult to determine reliably. Therefore, most investigators have measured cholinergic synthetic and metabolic enzymes or receptor binding as indices of cholinergic function. Region-specific decreases have been reported, but results have not always been consistent among different laboratories.

Choline acetyltransferase

In a study of human autopsy material, McGeer and McGeer (1975) reported that between the ages of 20 and 50, activity of the synthetic enzyme, choline acetyltransferase (CAT), declined 40-66% in certain cortical areas. Only slight declines were found in extrapyramidal and rhinencephalic regions. Moderate age-related declines in CAT in cortical

regions and a large decrease in hippocampus were reported in another human investigation (Perry, Perry, Gibson, Blessed, & Tomlinson, 1977). Activity of CAT in the caudate nucleus was not significantly affected by aging. Bird and Iversen (1974) reported no change in CAT activity in the human putamen with aging.

Several investigators have reported no effect of aging on CAT activity in rat cortex (Meek, Bertilsson, Cheney, Zsilla, & Costa, 1977; Timiras & Vernadakis, 1972). However, Strong et al. (1980) reported a 30% decrease in 18-month-old rats and a 54% decrease in 26-month-old rats compared to 10-month-old animals. They also reported a 13% decrease in CAT activity in the striatum of 26-month-old vs. 10-month-old rats, but no change in hippocampus. Meek et al. examined CAT activity in nucleus accumbens, caudate nucleus, nucleus interpeduncularis, locus coeruleus, septum, and the hippocampus. They reported a 28% decrease in CAT in the caudate of 24-month-old rats compared to 35- to 45-day-old rats, but no change in any other region. McGeer, Fibiger, McGeer, and Wickson (1971) also reported a decrease with aging in CAT activity on the rat caudate, but Reis, Ross, and Joh (1977) found no difference between 3- and 26-month-old rats in caudate CAT activity. Decreased CAT activity with aging has been reported in the cerebellum with no change in the striatum in a comparison of 4- and 22-month-old rats (Morin & Wasterlain, 1980). However, Timiras and

Vernadakis (1972) found no difference in the cerebellum of 2-, 12-, and 20-month-old rats.

In the C57BL/6J mouse, Vijayan (1977) compared CAT activity of animals 3, 8, 16, and 24 months of age. He reported an age-related decrease in the hippocampus but no change in the cerebellum. Strong et al. (1980) found no age differences in hippocampal or cortical CAT in a comparison of 6-, 12-, and 30-month-old C57BL/6J mice. In the striatum they reported a 25% decrease in CAT activity between 30- and 6-month-old mice.

Acetylcholinesterase

Acetylcholinesterase (AChE), a metabolic enzyme for acetylcholine, is a less reliable indicator of cholinergic function than is CAT (Kuhar, 1976). Results from studies of the effect of aging on AChE activity have been as variable as those dealing with CAT.

McGeer and McGeer (1975) reported age-related declines in AChE activity in cortical regions of human brain, comparable to those they reported for CAT. However, Perry (1980) found no difference in AChE activity in human cortex with normal aging.

In the rat, Moudgil and Kanungo (1973) reported that AChE activity was highest in immature animals and decreased progressively with aging. Hollander and Barrows (1968) reported minor age-related decreases in activity of AChE in

rat forebrain and cortex. Since the largest declines occurred before 12 months of age, these investigators suggested that the change might be more related to development than to aging. Significant age-related decreases in AChE activity have been reported in the striatum and cerebellum of rats (Morin & Wasterlain, 1980). McGeer et al. (1971) found no effect of aging on AchE activity in the caudate nucleus or in the remainder of the rat brain.

A decrease in whole brain AChE activity in the C57BL/10 mouse after the age of 16 months has been reported (Ordy & Schjeide, 1973). Vijayan (1977) reported a significant decline in activity of AChE in C57BL/6J mouse hippocampus with aging, but no change in the cerebellum.

Choline uptake

Age-related alterations in brain uptake of choline have recently been reported. Hicks, Rolsten, Hsu, Schoolar, and Samorajski (1979) reported that the choline chloride brain uptake index was lower in 7 out of 17 regions examined in old rats. They suggested that the blood-brain barrier transport system for choline may decrease with age. High affinity choline uptake in hippocampus P_2 fraction of 24-month-old Fischer rats was 20% lower in capacity, compared to that of 6-month-old rats (Sherman, Dallob, Dean, Bartus, & Friedman, 1980). These investigators attributed their findings to a reduced number of transport sites and/or a cholinergic neuronal loss.

Receptor binding

Putative cholinergic muscarinic receptor function is generally evaluated by examining binding characteristics of radiolabeled ligands presumed to compete with endogenous ACh for receptor sites in brain tissue homogenates. Age-related decreases in binding of these ligands (e.g., [^{3}H] quinuclidinyl benzilate (QNB), [^{3}H]atropine, and [^{3}H]scopolamine) have been reported in both human and animal investigations.

Perry (1980) reported a significant age-related decline in [^{3}H]scopolamine binding in temporal cortex of mentally normal humans. Decreased [^{3}H]atropine binding with aging in human frontal cortex has also been reported (White, Hiley, Goodhardt, Carrasco, Keet, Williams, & Bowen, 1977). Interestingly, these investigators found decreased binding only in brains without evidence of morphological degeneration associated with Alzheimer's senile dementia. According to Perry's (1980) report, binding in brains from Alzheimer's disease patients was similar to that of young controls. Davies and Verth (1977) reported no decrease in [^{3}H]QNB binding in hippocampus of Alzheimer's disease cases. However, Reisine, Yamamura, Bird, Spokes, and Enna (1978) reported a significant decrease in [^{3}H]QNB binding in hippocampus, but not caudate, frontal cortex, or putamen of Alzheimer's patients.

The human studies raise an interesting issue. Most data indicate that binding of cholinergic ligands is not

altered in Alzheimer's disease. Instead, this form of dementia appears to be related to losses of afferent processes, as evidenced by decreased levels of CAT and the presence of senile plaques and neurofibrillary tangles (Perry, 1980). This dissociation between cholinergic changes accompanying Alzheimer's disease and normal aging suggests that the dementia of Alzheimer's disease may be of a different etiology from the memory loss which occurs to a variable extent with normal aging. The significance of cholinergic changes, including receptor losses, which are associated with normal aging has yet to be determined.

Animal studies have corroborated age-related receptor decreases, although not all investigators report decreases in the same brain regions. James and Kanungo (1976) reported data on 20-month-old male and female Wistar rats indicating that [^{3}H]atropine binding in cerebral cortex decreased approximately 50% in comparison to 2-month-old animals. Binding in 10-month-old animals was intermediate between the other two age groups. In cerebellar cortex, however, binding appeared to increase from 2 to 10 months of age, and then decreased at 20 months to a level similar to that of 2-month-old animals.

Morin and Wasterlain (1980) reported significant decreases in [^{3}H]QNB binding in the striatum and cerebellum of 22-month-old female Long-Evans rats compared to 4-month-old rats. Specific [^{3}H]QNB binding was decreased by 28% in the striatum and 25% in the cerebellum with no change

occurring in the hippocampus, amygdala, hypothalamus, or cerebral cortex. Scatchard analysis of the data indicated that the decreases were due to a change in the number of binding sites and that binding affinity was unchanged. The absence of an aging effect in cerebral cortex stands in obvious contradiction to the data of James and Kanungo on cerebral cortex.

A significant 16% decrease in [^{3}H]QNB binding has been reported in the dorsal hippocampus of 26- to 29-month-old rats compared to 6- to 9-month-old animals (Lippa, Critchett, Bartus, Harrington, & Pelham, 1979). The investigators similarly reported a decrease in the number of binding sites with no change in affinity. Lippa et al. also found a 50% reduction in old animals in the response of hippocampal pyramidal cells to iontophoretically applied ACh.

Freund (1980) reported binding data on whole mouse brain (not including the cerebellum). No decrease in [^{3}H]QNB binding in C57BL/6J mice was observed until the age of 18 months. In comparison to 5-month-old animals, binding was decreased by 9% at 26 months and 22% at 30 months. Again, Scatchard analysis revealed that the change was due to a decrease in receptor density rather than a change in affinity. Freund also examined binding in young mice which had been deliberately underfed as a control for the effects of nonspecific illness and severe weight loss. This treatment had no effect on whole brain [^{3}H]QNB binding.

One regional analysis of [^{3}H]QNB binding in aged C57BL/6J mice has recently been reported. Strong et al. (1980) compared [^{3}H]QNB binding in the striatum, cerebral cortex, and hippocampus of 6-, 12-, and 30-month-old mice. No significant difference was found between 6- and 12-month-old mice in any region. Comparing 6- and 30-month-old mice, a significant 34% reduction in cortex and a significant 30% reduction in striatum were reported in the older mice. A 27% decrease in the hippocampus was not statistically significant. Scatchard analysis of binding in cerebral cortex indicated a loss of binding sites with no change in receptor affinity in the aged animals.

In conclusion, decreases in binding of labeled cholinergic ligands appear to accompany normal aging in human and rodent brains. Decreased receptor density with no change in receptor affinity has been a relatively consistent finding in the aging literature. Regarding specific regions of brain particularly affected by receptor losses, evidence is preliminary, but animal data indicate that the striatum may be more affected by aging than other regions. In the human, decreases are apparent in cortical regions. Animal data are conflicting for cerebral cortex, hippocampus, and cerebellum. The need for more conclusive regional analyses is clear.

Pharmacological Manipulations of Cholinergic Function in Aged Organisms

Evidence implicating the cholinergic system in memory function and research indicating age-related changes in cholinergic function have stimulated interest in manipulating the cholinergic system to possibly ameliorate memory deficits associated with aging. Drachman and Leavitt (1974) demonstrated that treatment of young subjects with the anticholinergic agent, scopolamine, mimicked the effect of aging on performance. Based on this and other studies, Davis and Yamamura (1978) suggested in a review paper on the cholinergic system and memory disorders that cholinergic-potentiating drugs might benefit elderly subjects with memory deficits.

Animal experiments have provided some support for this possibility. Bartus (1979) investigated the effect of physostigmine on short-term memory (STM) in young (3-7 years) and old (18+ years) rhesus monkeys. In young monkeys low doses of the drug had no effect on STM, moderate doses resulted in some improvement, and at the highest dose performance was impaired. Prior to drug testing, all aged monkeys had shown memory impairments. Although old monkeys improved at the same general doses of physostigmine, there was more variability in their response to the drug. Old monkeys in some cases were facilitated by doses which disrupted young monkeys' performance and vice versa. Drug response in individual animals within the aged group was not related to the severity of their pre-drug performance deficits.

More promising results were obtained in an investigation of the effect of dietary manipulations of choline, the precursor for acetylcholine, on retention performance of C57BL/6J mice (Bartus, Dean, Goas, & Lippa, 1980). Mice 8.5 months of age were given either choline-enriched or choline-deficient diets for 4.5 months and subsequently trained and tested on a step-through passive avoidance task. In a separate group of animals on normal diets, a minimal deficit was reported in performance of 13-month-old animals (compared to 3-month-old animals), and a more severe deficit at 24 months. At 13 months of age the choline-enriched group performed as well as 3-month-old mice, while the choline-deficient group showed retention deficits similar to those of 23-month-old mice. However, choline manipulations of old mice already deficient in retention were not investigated in this study.

The effect of choline has been tested on elderly human patients with known memory deficits (Mohs, Davis, Tinklenberg, Hollister, Yesavage, & Kopell, 1979). In this study 8 elderly patients with mild memory impairment were administered choline chloride for 7 days and a placebo before and after drug treatment, and tested for memory storage and retrieval under each condition. Although one patient improved considerably on the retrieval test during choline treatment, the drug had no effect on storage or retrieval in the other subjects.

In view of conflicting results, conclusions as to the usefulness of cholinergic manipulations would be premature. The Bartus (1979) study suggested that physostigmine may not be a reliable therapeutic agent in age-related short-term memory dysfunction. Chronic choline treatment appeared to prevent LTM retention deficits in mice, and choline deprivation appeared to exacerbate them (Bartus et al., 1980). These results were interpreted cautiously. Bartus et al. pointed out that they had not demonstrated neurochemically that cholinergic function had been altered in their animals. They also suggested that choline could be affecting other neurotransmitter systems, for example, increasing the synthesis of β-adrenergic receptors. It remains to be seen if choline enrichment can reverse deficits once they have developed in aged organisms. The investigation by Mohs et al. (1979) of choline treatment in aged humans produced disappointing results. These investigators suggested that a longer duration of treatment might be necessary to produce improvement. Further investigation will be necessary to determine the potential and limitations of cholinergic manipulations in regard to age-related memory dysfunction. The lack of conclusive positive evidence to date suggests that other manipulations, pharmacological or behavioral, might be equally or more beneficial toward this end. For this reason a comparison of the effects of exposure to "enriched" or "impoverished" environments, as discussed below, was incorporated into the present investigation.

Environmental Enrichment and Aging

Although the environmental enrichment literature is large and dates back to the early 1960's, the number of studies dealing with aged organisms is very small. Riege (1971) conducted one such study using 1-year-old rats (more appropriately considered middle-aged than aged animals). He did not include young controls in this study. Instead, he compared his results with those of Rosenzweig and Bennett on weanling and young adult rats.

In this study the enriched condition (EC) consisted of group housing in a large cage with a variety of play objects that were changed daily and an additional 30 min daily testing period in a Hebb-Williams maze for each animal. In the standard colony condition (SC) animals were housed 2 per cage without play objects or the maze testing experience. Animals in the impoverished condition (IC) were housed individually in wire-mesh cages in a semi-darkened room. Differential housing was maintained for 30, 60, or 90 days. The traditional measures, whole brain weight, cortical weight, acetylcholinesterase (AChE) activity and cholinesterase (ChE) activity were taken following several behavioral evaluations.

The results indicated that increases in brain weight, cortical weight, and occipital total AChE and ChE activity in year-old EC rats compared to year-old IC rats were comparable to EC--IC differences in younger rats. However, although 30 days produced a maximal effect in young animals,

older animals appeared to benefit from a longer duration (60 or 90 days) of the EC treatment. The year-old EC animals also demonstrated better performance on an agility test and a Lashley III maze than did IC animals.

Cummins, Walsh, Budtz-Olsen, Konstantinos, and Horsfall (1973) conducted a somewhat different study. Their animals were placed in differential environments at weaning, but remained there for 509 days, until they were approximately 18 months of age. After this period animals in the EC and IC groups (no SC condition was included) were subdivided into two groups, one group simply remained in its respective environment and the other group was tested for 21 days on a Hebb-Williams maze. Therefore, Cummins et al. compared four treatment conditions: enriched, non-tested; enriched, tested; isolated, non-tested; and isolated, tested.

The EC-IC comparisons yielded predictable results. EC animals had heavier brain weights (a 3% increase) than IC animals, and EC animals performed better on the Hebb-Williams maze than did IC animals. However, the results of an analysis of the effect of testing were somewhat surprising. IC animals that were tested on the maze demonstrated a 3% increase in brain weight compared to IC non-tested animals. This finding indicated that the effect of 21 days of maze testing on previously isolated animals was comparable to that of nearly 18 months of the enriched environment condition. Brain weights of EC animals, on the other hand were not affected by the testing experience. These findings

led Cummins et al. to conclude that there might be a ceiling effect on environmentally-induced changes.

The available evidence appears to indicate that 12- and 18-month-old rats remain susceptible to the beneficial effects of EC housing and/or behavioral testing. Based on these two studies the optimal duration of such treatment is unclear. In the Riege study 90 days appeared to maximize differences. However, Cummins et al. showed that the effect of 21 days of testing was comparable to 18 months of EC treatment. This issue has yet to be resolved. Since brain weight and AChE-ChE activity are relatively crude measures of unknown significance, it would be interesting to examine other neuroanatomical and/or neurochemical measures in studies of this sort. Nevertheless, these investigations do suggest that environmental manipulations may induce both behavioral and neurobiological changes in older animals.

Rationale

A decline in memory function associated with aging has been documented above from both the human and animal experimental literature. Neurochemical research on aging has been reviewed indicating that the central cholinergic system is affected by aging, as evidenced by alterations in synthetic and metabolic enzymes and receptor binding. Because neuropharmacological research has strongly implicated the cholinergic system in memory function, a cholinergic hypothesis of age-related memory loss has achieved increasing popularity.

However, evidence directly supporting this hypothesis is limited. To date behavioral and neurochemical measures have usually been made on separate groups of animals. Strong et al. (1980) who measured behavior and cholinergic function in the same animals, 6, 12, or 30 months of age, found performance deficits at 12 months, but no significant loss of CAT or decrease in [^{3}H]QNB binding until 30 months of age. Attempts to facilitate memory in aged organisms by manipulating the cholinergic system have met with limited success in animals and virtually no success in humans.

The investigation which follows was designed principally to test the cholinergic hypothesis of age-related memory loss. The effect of age on several behavioral measures and regional [^{3}H]QNB binding was examined in the same animals in Experiment 1. Age group comparisons were expanded to include 4-, 8-, 15-, 20-, and 25-month-old mice, rather than restricting comparisons to 2 or 3 ages which is common in the aging literature. In addition to comparing the time course of behavioral changes and binding changes, the present investigation aimed to determine the relationship between performance scores and binding values in individual animals.

The behavioral tasks, spontaneous alternation, step-through passive avoidance, and step-down passive avoidance, were chosen for the following reasons. Spontaneous alternation is a behavior particularly sensitive to damage to the

hippocampus (e.g., O'Keefe & Nadel, 1978), and the hippocampus appears on anatomical and physiological grounds to be particularly susceptible to aging effects (see Kubanis & Zornetzer, 1981). Much of the evidence for cholinergic effects on memory has involved passive (inhibitory) avoidance tasks (e.g., Carlton, 1968). Furthermore, age-related deficits have been reported on step-through passive avoidance in C57BL/6J mice (Bartus et al., 1980; Strong et al., 1980) and on step-down passive avoidance in an outbred (Swiss) mouse strain (Kubanis et al., 1981). By examining retention at both very short and longer post-training intervals, the relative importance of acquisition vs. retention effects could be determined. This distinction, obviously important in view of results of human research on aging and memory, has been neglected in much of the animal research.

As a measure of cholinergic function, [^{3}H]QNB binding was chosen because it is relatively unaffected by diurnal fluctuation and post-mortem changes (Freund, 1980). Results of studies of age-related changes in binding in human and rat brain have been conflicting, and data on [^{3}H]QNB binding in the C57BL/6J mouse are sparse. A comprehensive regional analysis of [^{3}H]QNB binding in C57BL/6J mice of a wide variety of ages was viewed as a needed contribution to the aging field.

Finally, a comparison of the effect of enriched vs. impoverished environments was incorporated into Experiment 1. It was hypothesized that exposure to an enriched environment

might facilitate memory in animals that would otherwise be memory-deficient. Although this approach has not been reported to date using aged animals, evidence is available on the effect of differential housing on passive avoidance performance in young animals. Greenough, Fulcher, Yuwiler, and Geller (1970) reported that retention of step-through passive avoidance was superior in DBA2J mice exposed to an enriched environment, compared to impoverished mice. Another study indicated that social isolation and/or perceptual deprivation disrupted 24-hr retention in rats of step-down passive avoidance (Gardner, Boitano, Mancino, D'Amico, and Gardner, 1975). However, Parsons and Spear (1972) reported that differential housing had no effect on step-through performance of rats. Freeman and Ray (1972) reported that "isolated" rats (2 per cage) performed better than animals housed in an enriched environment on 24-hr retention of step-through passive avoidance.

The effect of differential environments on passive avoidance performance is not clear. Nevertheless, considering the relative ineffectiveness of cholinergic pharmacological manipulations, a behavioral approach appeared to be a reasonable alternate possibility for improving memory in aged organisms. In addition to evaluating the effect of environment on behavior, the effect of this manipulation on [^{3}H]QNB binding was examined.

The plastic capability of the cholinergic system in the mouse was investigated in Experiment 2. Chronic treatment

with the cholinergic muscarinic antagonist, scopolamine, has been reported to increase putative cholinergic receptor density in the hippocampus of young rats (Ben-Barak & Dudai, 1980). Greenberg and Weiss (1978, 1979), who investigated the effect of age on plastic capabilites of the β-adrenergic system, reported that environmental and pharmacological manipulations which increased [^{3}H]dihydroalprenolol (a putative β-adrenergic receptor ligand) binding in young rats did not increase binding in old rats. Experiment 2 was included to determine (1) whether the augmented receptor density response to scopolamine reported in rats also occurs in mice, and if so, (2) whether the effect is restricted to the hippocampus, and (3) whether the capability for this response changes as a function of age.

Experiment 3 was designed to attempt to replicate and extend the only other regional analysis of [^{3}H]QNB binding in aged C57BL/6J mice in the aging literature (Strong et al., 1980). In view of evidence suggesting that homeostatic control of arousal and anxiety declines with aging (see Kubanis & Zornetzer, 1981, for a review) binding of [^{3}H]flunitrazepam (FNP), a putative benzodiazepine receptor ligand, was also examined in Experiment 3. Endogenous benzodiazepine-like compounds have been implicated in animal models of anxiety and fear (e.g., Martin, 1980). The effect of aging on [^{3}H]FNP binding has not been reported in the literature. Therefore, regional analyses of [^{3}H]QNB and [^{3}H]FNP binding were performed on 4- and 29-month-old mice. This extreme

age comparison was chosen to maximize possible age differences in binding.

In summary, these experiments were intended to provide a multi-faceted investigation of age-related changes in memory and [^{3}H]QNB and [^{3}H]FNP binding in the C57BL/6 mouse. In Experiment 1 the effect of aging on several behavioral measures was examined and compared to the effect of aging on regional [^{3}H]QNB binding in the brains of the same animals. The effect of differential environments was examined on both behavior and receptor binding. Plastic capability of the brain cholinergic system was investigated by a pharmacological manipulation in Experiment 2. Receptor binding comparisons of [^{3}H]QNB and [^{3}H]FNP were performed on extreme age groups in Experiment 3, thereby extending the findings of the first experiment and increasing the generality of this work to previous reports in the literature.

CHAPTER II

METHODS

Experiment 1

Behavioral Comparisons

In this experiment the effects of age and differential environments on several behavioral measures were examined. Following 1 month of exposure to either an enriched or impoverished environment, animals were tested on spontaneous alternation, step-through passive avoidance, and step-down passive avoidance. All animals were tested first on spontaneous alternation. The order of the two passive avoidance tasks was counterbalanced, such that half of the animals were tested on step-through prior to step-down, and half on step-down prior to step-through. This was done to prevent possible facilitative or disruptive order effects. Previously published research on Swiss mice (Kubanis et al., 1981) and pilot studies on C57BL/6 mice revealed no effect of age on shock sensitivity thresholds. Therefore, this was not a complicating factor in the experiments involving footshock.

Subjects

Male C57BL/6 mice of different ages were subjects in Experiment 1. At the onset of this experiment, mice were the following ages: 3 months, 7 months, 14 months, 19 months, and 24 months. Two hundred mice in total were subjects, 40 in each age group. The 14- and 19-month-old animals were obtained from Jackson Laboratories as retired breeders and were subsequently raised in my animal facility. The 3- and 24-month-old animals were obtained from Charles River Breeders. Half of the 7-month-old animals were obtained from Jackson Laboratories and were raised in my facility, and the other half were retired breeders obtained from Charles River Breeders. The C57BL/6J strain refers to animals from Jackson Laboratories exclusively; therefore, these are referred to as C57BL/6 mice. Obtaining mice from two different breeders was necessary because of the limited availability of aged animals.

Housing conditions and environmental manipulations

Animals were housed for a period of 1 month prior to behavioral testing and during testing in either an enriched condition (EC) or impoverished condition (IC) as described below.

Twenty animals in each age group were placed in the EC environment. They were housed 10 per cage in large metal cages (60 cm x 45 cm x 26 cm). These cages contained a variety of objects to provide perceptual and sensorimotor

stimulation including ladders, sandboxes, ramps, tunnels, running wheels, mirrors, and colored movable toys of different sizes. A unique enriched environment was set up in each of the 10 cages. Every 2 days the environments were rotated from cage to cage in order to expose all animals to each of the environments and to provide continually changing environments.

The remaining 20 animals in each age group were placed in the IC environment. These animals were housed individually in metal cages (24 cm x 18 cm x 17 cm). No exposure to other mice was allowed during this period, and the cages contained no play objects. Racks of cages were arranged so that animals could see only a wall or divider separating them from an adjacent rack of cages.

All animals (EC and IC) were handled only during cage cleaning once a week. Food and water were provided ad libitum, room temperature was approximately 23°C, and a 7 am to 7 pm light-dark cycle was maintained. Cage floors were covered with wood shavings. Animals in both conditions were housed in the same room, but IC animals were confined to a far corner of the room and separated by a divider to prevent unintentional environmental stimulation.

Spontaneous alternation

Apparatus. The apparatus consisted of a T-maze constructed of white Plexiglas. The start alley measured 51 cm long x 7 cm wide x 10 cm high. Perpendicular to the

start alley, the left and right side arms measured 15 cm long x 7 cm wide x 10 cm high.

Procedure. Mice were tested using a single-trial method which is considered preferable to multi-trial methods (O'Keefe & Nadel, 1978). In a dimly-lit testing room each mouse was initially placed in the start alley and allowed to enter whichever side arm it preferred. After entry the mouse was confined to the arm for 5 sec, removed, and placed in a holding cage for 45 sec prior to re-testing. On the basis of directional choice on the second trial, each animal's behavior was classified as alternating (choosing the opposite arm) or not alternating (choosing the same arm). The apparatus was cleaned with 50% ethyl alcohol between animals.

Statistical analysis. The overall frequency of alternation was analyzed by a one-way χ^2 test to determine whether it differed significantly from chance. To determine the effects of age and environment on spontaneous alternation, two-way χ^2 tests were performed.

Step-through passive avoidance

Apparatus. The step-through apparatus was a trough-shaped chamber consisting of a smaller (7 cm long) outside light compartment and a longer (22 cm long) inside dark compartment constructed of Plexiglas. A bright lamp illuminated the outside compartment. The floor of the inside compartment consisted of 2 metal plates through which (DC)

footshock was delivered from a Scientific Products shock source.

Procedure. During training each mouse was placed in the light compartment of the chamber facing away from the entrance to the dark compartment, and initial step-through latency (STL) was recorded. After the animal completely entered the dark compartment, a footshock (300 μA) was delivered to the floor of the apparatus. Footshock continued until the animal escaped to the light compartment at which time it was returned to its home cage. Any animal that did not enter the dark compartment in 300 sec was assigned an initial STL of 300 and eliminated from further testing.

Animals were divided randomly into two groups for testing purposes. Approximately 2/3 of the animals from each age group were tested for retention 5 days following training. The remaining 1/3 were tested 2 hr after training. The 2-hr group was included to examine the effects of age and environment on acquisition of this task.

Testing was identical to training except that footshock was not delivered. Step-through latency difference scores were calculated by subtracting each animal's initial STL from its testing STL. An arbitrary ceiling of 600 sec was imposed on the STL difference scores.

Statistical analysis. The effects of age and environment on STL difference scores were analyzed using nonparametric one-way Kruskal-Wallis tests. Similar analyses were

performed to evaluate the effects of age and environment on initial STL's. Where statistically significant overall effects were obtained, Mann-Whitney U tests were used to compare specific age groups.

Step-down passive avoidance

Apparatus. The step-down apparatus consisted of a box 18 cm long x 13 cm wide x 16 cm high. The long walls were constructed of clear Plexiglas and the end walls and top were aluminum painted black. An aluminum platform 6 cm x 6 cm was attached to one of the end walls approximately 2.5 cm above the floor of the apparatus. Footshock was delivered to the grid floor from a Lafayette (AC) shock source by activating a foot pedal.

Procedure. A single-trial procedure was utilized in these experiments, since reliable age-related deficits in Swiss mice have been found using this procedure (Kubanis et al., 1981). During training each mouse was placed on the platform and initial step-down latency (SDL) was recorded. After all four paws contacted the grid floor, footshock was delivered until the animal escaped to the platform, at which time it was removed from the apparatus and returned to its home cage.

Animals were tested either 24 hr or 2 hr after training. Approximately 2/3 of the animals were tested for retention after 24 hr on the basis of previously reported deficits in aged Swiss mice at this training-test interval (Kubanis et

al., 1981). The remaining 1/3 were tested after 2 hr to evaluate acquisition, as in the step-through experiment.

Testing was identical to training except for the absence of footshock. Step-down latency difference scores were calculated by subtracting initial SDL from testing SDL. A 600 sec ceiling was imposed on the difference scores.

Statistical analysis. The effects of age and environment on SDL difference scores were analyzed using nonparametric one-way Kruskal-Wallis tests. Similar analyses were performed to evaluate the effect of age and environment on initial SDL's. Where statistically significant overall effects were obtained, Mann-Whitney U tests were used to compare specific age groups.

Receptor Binding

Following behavioral testing, the effects of age and differential environments on binding of [^{3}H]QNB in the cerebral cortex, striatum, hippocampus, and cerebellum were investigated. In the comparison of environment, a third housing condition, the standard condition (SC), was included. Animals in the SC environment were group housed (6 per cage) in clear plastic cages 40 cm x 24 cm x 15 cm. No behavioral testing was performed on these animals. Therefore, they served as a control group for possible effects of the testing procedures on receptor binding.

Because behavioral data were available on the EC and IC animals, an additional variable, performance, was included in the binding studies. Animals were classified as either good or poor performers on the basis of their step-through and step-down difference scores. The best and worst performers in each age group were selected for this dichotomous classification.

Subjects

Subjects for the binding assays in Experiment 1 were male C57BL/6 mice of the following ages: 4 months, 8 months, 15 months, 20 months, and 25 months. The EC and IC mice had attained these approximate ages by the end of the behavioral comparisons. Animals in the SC condition were the same ages as the EC and IC animals, but had not been exposed to unusual housing conditions or behavioral testing. An equal number of animals were used in each age group. Eighty animals in total, 30 EC, 30 IC, and 20 SC, were used in the assays.

Dissection

Immediately following decapitation intact heads were sealed in plastic bags and placed in a -15°C freezer. After 24 hr the bags were transferred to a -60°C freezer for storage. Twenty-four hours prior to dissection they were returned to the -15°C freezer, and exactly 1 hr prior to dissection each brain was allowed to thaw to 6°C in a

refrigerator. It was determined during pilot experiments that this particular combination of freezing temperatures and durations yielded brain tissue most closely resembling fresh tissue for dissection.

After 1 hr of thawing, the brain was removed from the skull and placed on a glass plate over ice. A fiber optics light provided cool illumination during dissection under a dissecting microscope (American Optical). The procedure was briefly as follows: (1) the olfactory bulbs were removed and discarded, (2) the cerebral hemispheres were separated with a probe and a symmetrical section of cortex was removed from each side, exposing the hippocampus and striatum, (3) the striatum was teased out of the surrounding tissue on each side, (4) the hippocampus was removed bilaterally, and (5) the cerebellum was separated from the brainstem. As each structure was removed it was immediately weighed and placed into ice cold Tris (pH 7.4) for homogenization. The homogenates were frozen at -15°C for subsequent binding assays.

Determination of the effect of freezing

The procedure of freezing brains prior to dissection was adopted to increase the uniformity of conditions for each assay. It enabled the investigator to dissect on the same day every brain that was slated for a particular binding assay. Day to day variability in the dissection procedure, time of day at sacrifice, and order of dissection

of brains from different age groups and environments could thereby be effectively controlled.

Before adopting this technique a control experiment was performed to determine the effect of prior freezing on binding values. Twelve animals were included in this study, approximately 8 months of age. Six brains previously frozen and thawed according to the specifications described above and 6 fresh brains were extracted, weighed, and homogenized. Samples from the whole brain homogenates were then immediately subjected to the standard [^{3}H]QNB and [^{3}H]FNP binding procedures as described below. Tissue suspension samples were also taken from the homogenates for determination of protein.

Because the usual procedure involved dissecting brains and freezing the homogenates on one day and performing receptor binding assays on subsequent days, an additional condition was included in this study. Half of the homogenate from each brain was frozen, and binding of [^{3}H]QNB and [^{3}H]FNP was determined on the following day after thawing and resuspending the homogenates. Samples for protein determination were taken again for a second comparison.

Thus, this experiment provided a comparison of binding and protein values for fresh vs. frozen tissue. In addition, the effect of freezing homogenates prior to assay was evaluated, in case this additional freezing step affected the results of the assays.

[^{3}H]QNB binding assay

After resuspension of the homogenates, 100 µl aliquots were pipetted and diluted with 900 µl Na^+-K^+ phosphate buffer using a Micromedic System automatic pipette. Samples were prepared in triplicate for both total and nonspecific binding. One milliliter of a 2.18 nM/ml solution of [^{3}H]QNB in Na^+-K^+ phosphate buffer (approximately 8.5 X 10^4 cpm) was incubated with each sample for 1 hr to determine total binding. For determination of nonspecific binding, the [^{3}H]QNB was added in the presence of 100 µl of a 1 nM/100 µl solution of unlabeled QNB. In order to maintain a constant volume in the total and nonspecific binding samples, an additional 100 µl of buffer were added to the total binding samples.

All samples were filtered under vacuum pressure on Whatman GF/B filters and rinsed 3 times with 3.0 ml Na^+-K^+ phosphate buffer. Filters were transferred to scintillation vials, 0.5 ml Protosol was added, and the vials were heated at 55°C for 30 min. After cooling, 50 µl of glacial acetic acid and 10.0 ml of scintillation fluid (Econofluor) were added and the vials were vortexed thoroughly. Vials were stored for 48 hr to allow equilibration prior to counting in a liquid scintillation counter. On separate 100 µl aliquots of resuspended homogenate, protein was determined in triplicate according to the method of Lowry, Rosebrough, Farr, and Randall (1951).

[^{3}H]FNP binding assay

The procedure used for [^{3}H]FNP binding assay was similar to that used in the [^{3}H]QNB binding assay. Samples were prepared in triplicate as described above, except that Tris (pH 7.4) was used as buffer. One milliliter of an 8.62 nM/ml solution of [^{3}H]FNP in Tris buffer (approximately 6.5×10^5 cpm) was incubated with each sample for 30 min in an ice bath to determine total binding. For determination of nonspecific binding, the [^{3}H]FNP was added in the presence of 100 µl of a 10 nM/100 µl solution of clonazepam. To maintain a constant volume in total and nonspecific binding samples, 100 µl of buffer were added to the total binding samples.

The procedures for filtration, preparation of scintillation vials, scintillation counting, and protein determination were identical to the methods described in the [^{3}H]QNB binding assay, except that filters were rinsed 4 times with 3.0 ml of Tris buffer.

Statistical analysis

For both [^{3}H]QNB and [^{3}H]FNP, maximal specific binding was calculated by subtracting the mean cpm of radioactivity of the 3 nonspecific samples from the mean cpm of the 3 total binding samples. These data were expressed as fmol/mg protein as determined by the Lowry assay.

The effects of age and environment on [^{3}H]QNB binding were analyzed by two-way analysis of variance procedures

(ANOVA) for each brain region, cortex, striatum, hippocampus, and cerebellum. Duncan's multiple range test was used for specific comparisons in the event of significant overall F ratios. Initially 12 animals in each age group were included in the assays (4 EC, 4 IC, and 4 SC). For comparisons that were suggestive but not significant, 4 additional animals in each age group were assayed.

To analyze the relationship between performance and receptor binding, one-way ANOVA comparisons of [^{3}H]QNB binding in good performers vs. poor performers were calculated for each brain region. Because only the EC and IC animals were behaviorally tested, SC animals were not included in these analyses. Spearman rank correlation coefficients were also calculated to determine the degree of association in individual animals between difference scores on the passive avoidance tasks and regional binding of [^{3}H]QNB.

Autopsies

In order to be certain that possible differences in behavior or receptor binding between age groups were not due to the presence of disease conditions in older animals, post-mortem examinations were performed. Twenty of the 25- and 20-month-old animals and 10 of the animals in each of the other age groups were autopsied grossly. After sacrifice by cervical dislocation and decapitation, the peritoneal cavity was opened and all internal organs were examined

carefully. Each organ system was probed and examined superficially and then dissected with a scalpel to look for tumors, signs of inflammation, infection, enlargement, atrophy, or other abnormalities.

Experiment 2

In Experiment 2 the effect of chronic scopolamine administration on [^{3}H]QNB binding in the C57BL/6 mouse brain was examined. Because the augmented receptor density response had previously been reported only in the hippocampus of young rats, the present investigation examined [^{3}H]QNB binding in cerebral cortex as well as the hippocampus. A comparison of young and aged mice was included in this experiment.

Subjects

Male C57BL/6 mice 4 and 25 months of age were subjects in Experiment 2. All mice were obtained from Charles River Breeders. Animals were housed in the SC condition as described in Experiment 1. Fifteen 4-month-old mice and 10 25-month-old mice were used in this experiment.

Drug Dose and Administration

Pilot experiments revealed that the dose of scopolamine used by Ben-Barak and Dudai (1980) in rats (10 mg/kg for 7 days) did not alter [^{3}H]QNB binding in hippocampus of

C57BL/6 mice. Therefore, a much larger drug dose was administered in the present investigation. Animals were injected subcutaneously with 100 mg/kg scopolamine hydrobromide (in 0.9% saline) twice a day (8 am and 8 pm) for 4 days. After 4 days the dose was increased to 150 mg/kg twice a day. The last injection was administered at 8 am, 7 days after the first injection. A saline control group was injected with an equal volume of 0.9% saline on the same schedule as the drug-treated animals. An additional control group received no injections but was weighed daily along with the injected groups. Each treatment group contained the same ratio of young to old animals (approximately 3/2) so that possible age differences in [^{3}H]QNB binding could not confound treatment effects.

Activity Measurement

Locomotor activity of scopolamine-treated animals vs. controls was compared in order to determine whether this drug dose had any overt behavioral effects. Thirty minutes after injection, animals were placed in activity chambers (40 cm x 24 cm x 15 cm) and photocell crossings were automatically counted for a period of 1 hr. Since there were 4 boxes, 4 animals were tested each day until all animals had been tested once.

Receptor Binding

All animals were sacrificed by cervical dislocation and decapitation 24 hr after the last injections were administered. The 24 hr interval was allowed to prevent any acute effect of the drug from interfering with the [^{3}H]QNB binding assay. The procedures used for dissection, tissue preparation, and the [^{3}H]QNB binding assay were identical to the methods described in Experiment 1. In this experiment [^{3}H]QNB binding was examined in the hippocampus and cerebral cortex.

Statistical Analysis

Locomotor activity

The total number of activity counts recorded in 1 hr was used as a measure of locomotor activity for each animal. Drug treatment and aging effects on locomotor activity were analyzed using a two-way ANOVA procedure.

[^{3}H]QNB binding

The [^{3}H]QNB binding data for hippocampus and cerebral cortex were calculated as described in Experiment 1 and expressed as fmol/mg protein. For each region a two-way ANOVA procedure was used to determine the effects of drug treatment and age on maximal [^{3}H]QNB binding.

Autopsies

All animals in this experiment were autopsied grossly for signs of pathology. The method used for autopsies was described in Experiment 1.

Experiment 3

In Experiment 3 a regional analysis of [^{3}H]QNB binding and [^{3}H]FNP binding was performed on animals from 2 extreme age groups. The oldest animals included in Experiment 1 were 25 months of age. Therefore, in this experiment binding in 4-month-old mice was compared to binding in 29-month-old mice. Average longevity of C57BL/6J mice is 28-30 months (Finch, 1977).

Subjects

Subjects in Experiment 3 were female C57BL/6J mice 4 and 29 months of age. All mice were obtained from Jackson Laboratories at 7-8 weeks of age and were reared under identical conditions in the Veterans Administration Medical Center animal facilities. Housing was comparable to the SC condition described in Experiment 1. Animals were group housed (8 per cage) and were not exposed to any behavioral testing procedures. Eight 4-month-old mice and 8 29-month-old mice were included in this experiment.

Receptor Binding

The methods used for sacrifice, dissection, tissue preparation, and the [^{3}H]QNB and [^{3}H]FNP binding assays were described in detail in Experiment 1. Identical procedures were used in this experiment. Both [^{3}H]QNB and [^{3}H]FNP binding were examined in all four brain regions: cerebral cortex, striatum, hippocampus, and cerebellum. In addition, Scatchard analyses were performed (Bennett, 1978) on those regions where significant age effects were found for binding of [^{3}H]QNB and/or [^{3}H]FNP.

Statistical Analysis

Binding data for [^{3}H]QNB and [^{3}H]FNP were calculated as described in Experiment 1 and expressed as fmol/mg protein. Since there were only 2 age groups compared in this experiment, t tests were used to analyze the effect of age on [^{3}H]QNB binding and [^{3}H]FNP binding in each brain region.

Autopsies

All animals in this experiment were autopsied grossly for signs of pathology. The method used for autopsies was described in Experiment 1.

CHAPTER III

RESULTS

Experiment 1

Behavioral Comparisons

Spontaneous alternation

The overall frequency of alternation in all animals tested (n = 181) was 67.4%. A one-way χ^2 test indicated that this differed significantly from a chance level of alternation $\chi^2(1) = 21.24$, $p < 0.001$. Table 1 shows the frequency of alternation in each age group and the EC and IC comparison. Although there appeared to be a slight decrease in alternation with increasing age, a two-way χ^2 test indicated that this difference was not statistically significant χ^2 (4) = 4.86, $p > 0.30$. A two-way χ^2 test indicated no difference between EC animals and IC animals in frequency of spontaneous alternation χ^2 (1) = 0.19, $p > 0.60$.

Step-through passive avoidance

Overall effects of age and environment on STL difference scores and initial STLs were analyzed using the Kruskal-Wallis procedure. Results are expressed as the χ^2 approximation for the value of H. The Mann-Whitney U test

TABLE 1

FREQUENCY OF SPONTANEOUS ALTERNATION
COMPARED BY AGE AND ENVIRONMENT

Age (months)	(n)	Frequency of Alternation
4	(38)	79.0%
8	(36)	72.2%
15	(38)	60.5%
20	(36)	58.3%
25	(33)	66.7%

Environment	(n)	Frequency of Alternation
EC	(87)	69.0%
IC	(94)	66.0%

was used for individual comparisons after significant main effects. Results of this test are expressed as the normal approximation (Z) for the value of U.

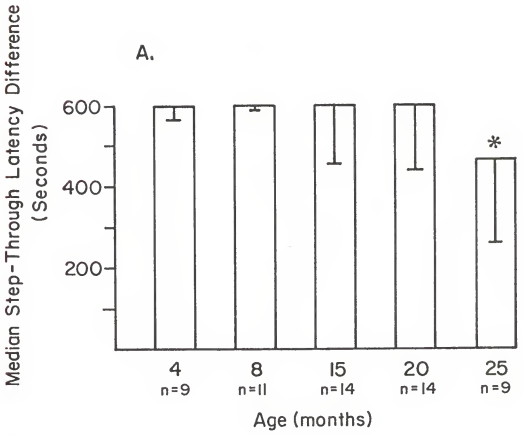
Analysis of the effect of age on step-through passive avoidance. In the 2-hr acquisition experiment a Kruskal-Wallis test indicated a significant effect of age on STL difference scores χ^2 (4) = 10.16, $p < 0.04$. Figure 1(A) shows the median STL difference scores for all age groups. Table 2 shows the probability values of individual comparisons using Mann-Whitney U tests. It demonstrates that STL difference scores of 25-month-old mice were significantly lower than those of 4-, 8-, or 15-month-old mice. A Kruskal-Wallis test revealed no significant effect of age on initial STLs in the 2-hr acquisition experiment χ^2 (4) = 7.94, $p > 0.09$.

The effect of age on STL difference scores in the 5-day retention experiment was statistically significant χ^2 (4) = 24.89, $p < 0.0001$. Figure 1(B) shows the median STL difference scores for all age groups. Table 2 shows the probability values of individual comparisons using Mann-Whitney U tests. It demonstrates that, in general, performance of the 15-, 20-, and 25-month-old groups was impaired relative to the 4- and 8-month-old groups. However, there appeared to be a trend toward relative preservation of performance in the 25-month-old groups compared to the 20-month-old group ($p < 0.07$). There was no significant effect of age on

Figure 1. Age comparison of performance on step-through passive avoidance.

A. Bar graph of median STL difference scores (+ interquartile deviation) by age, using a 2-hr training-test interval. Medians for 4-, 8-, 15-, 20-, and 25-month-old groups are 600, 600, 600, 600, and 464 sec, respectively. Asterisk indicates significantly different from 4-, 8-, and 15-month-old groups.

B. Bar graph of median STL difference scores (+ interquartile deviation) by age, using a 5-day training-test interval. Medians for 4-, 8-, 15-, 20-, and 25-month-old groups are 600, 600, 367, 252, and 477 sec, respectively. Asterisk indicates significantly different from 4-month-old group.


A.
Median Step-Through Latency Difference (Seconds)
600
400
200
*
4
n=9
8
n=11
15
n=14
20
n=14
25
n=9
Age (months)

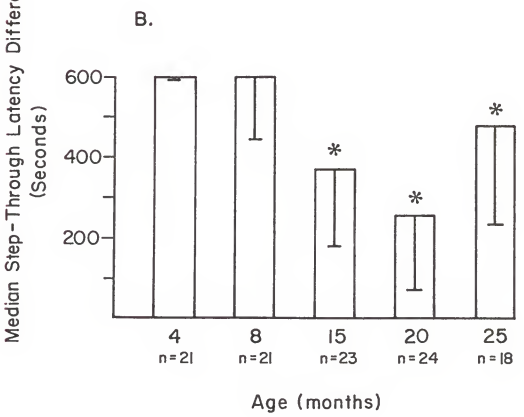

B.
Median Step-Through Latency Difference (Seconds)
600
400
200
*
*
*
4
n=21
8
n=21
15
n=23
20
n=24
25
n=18
Age (months)

TABLE 2

PROBABILITY VALUES FOR INDIVIDUAL COMPARISONS OF AGE GROUPS ON STL DIFFERENCE SCORES

2-Hr Acquisition Group

Months	4	8	15	20	25
4		0.59	0.61	0.57	0.02*
8			0.23	0.22	0.002*
15				0.85	0.04*
20					0.06
25					

5-Day Retention Group

Months	4	8	15	20	25
4		0.15	0.0006*	0.0001*	0.02*
8			0.05*	0.002*	0.17
15				0.14	0.48
20					0.06
25					

*$p \leq 0.05$

initial STLs in the 5-day retention experiment χ^2 (4) = 1.05, $p > 0.90$. The median initial STL was 27 sec.

Analysis of the effect of environment on step-through passive avoidance. Statistical comparison of EC and IC animals indicated no effect of environment on STL difference scores in the 2-hr acquisition experiment χ^2 (1) = 0.84, $p > 0.35$. Median 2-hr STL difference scores for both the EC group (n = 30) and the IC group (n = 27) were 600 sec. There was no significant difference between initial STLs of EC and IC animals in the 2-hr experiment.

In the 5-day step-through retention experiment the effect of environment on STL difference scores was not statistically significant χ^2 (1) = 0.40, $p > 0.52$. Median STL difference scores were 432 sec and 540 sec for the EC group (n = 53) and IC group (n = 53), respectively. The effect of environment on initial STLs in this experiment was statistically significant χ^2 (1) = 10.92, $p < 0.001$. Median initial STLs were 16 sec and 31 sec for the EC and IC groups, respectively.

Step-down passive avoidance

Step-down passive avoidance data were analyzed as described above for step-through passive avoidance. Therefore, results of the Kruskal-Wallis procedure are expressed as χ^2 values and Mann-Whitney U test results are expressed as Z values.

Analysis of the effect of age on step-down passive avoidance. In the 2-hr acquisition experiment the effect of age on SDL difference scores was not statistically significant χ^2 (4) = 3.45, $p > 0.48$. Figure 2(A) shows the median SDL difference scores for all age groups. Analysis of the effect of age on initial SDLs in this experiment revealed no significant difference χ^2 (4) = 4.38, $p > 0.35$. The median initial SDL was 9 sec.

In the 24-hr step-down retention experiment the effect of age on SDL difference scores was statistically significant χ^2 (4) = 16.30, $p < 0.003$. Figure 2(B) shows the median SDL difference scores for all age groups. Table 3 shows the probability values of individual comparisons using Mann-Whitney U tests. It demonstrates that SDL difference scores of 15-, 20-, and 25-month-old animals were significantly lower than those of 4-month-old animals. A comparison of 8- and 20-month-old animals' SDL difference scores was also significant.

In this experiment the effect of age on initial SDLs was statistically significant χ^2 (4) = 10.45, $p < 0.04$. Median initial SDLs were 30 sec, 9 sec, 11 sec, 4 sec, and 4 sec for the groups 4, 8, 15, 20, and 25 months of age, respectively. It is unlikely that this difference could account for the significant effect of age on 24-hr SDL difference scores described above. Since SDL difference scores are calculated by subtracting initial SDL from testing SDL, the longer initial SDLs in the 4-month-old groups would

Figure 2. Age comparison of performance on step-down passive avoidance.

A. Bar graph of median SDL difference scores (± interquartile deviation) by age, using a 2-hr training-test interval. Medians for 4- 8-, 15-, 20-, and 25-month-old groups are 600, 600, 470, 299, and 396 sec, respectively.

B. Bar graph of median SDL difference scores (± interquartile deviation) by age, using a 24-hr training-test interval. Medians for 4-, 8-, 15-, 20-, and 25-month-old groups are 600, 533, 297, 191, and 367 sec, respectively. Asterisk indicates significantly different from 4-month-old group.

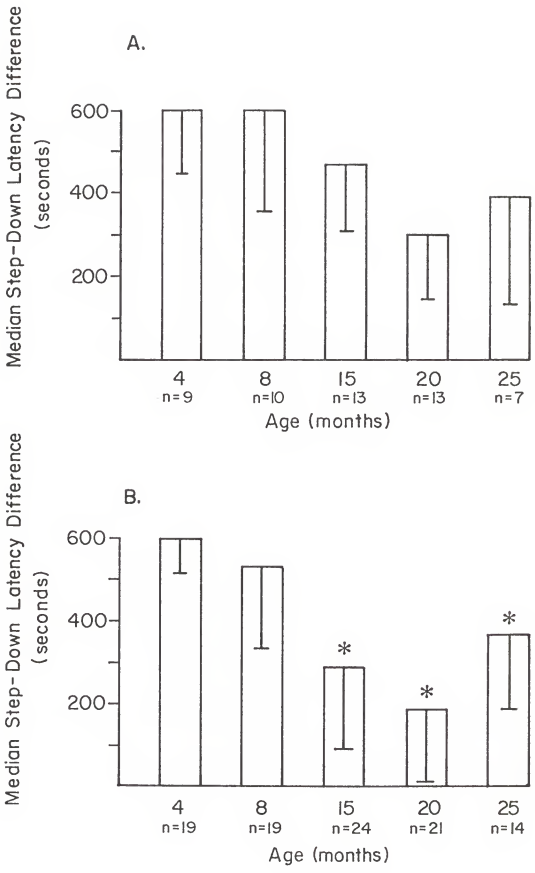

A.
Median Step-Down Latency Difference (seconds)
600
400
200
4
n=9
8
n=10
15
n=13
20
n=13
25
n=7
Age (months)
B.
Median Step-Down Latency Difference (seconds)
600
400
200
*
*
*
4
n=19
8
n=19
15
n=24
20
n=21
25
n=14
Age (months)

TABLE 3

PROBABILITY VALUES FOR INDIVIDUAL COMPARISONS OF AGE GROUPS ON SDL DIFFERENCE SCORES

24-Hr Retention Group

Months	4	8	15	20	25
4		0.20	0.004*	0.0004*	0.01*
8			0.14	0.05*	0.17
15				0.20	0.71
20					0.15
25					

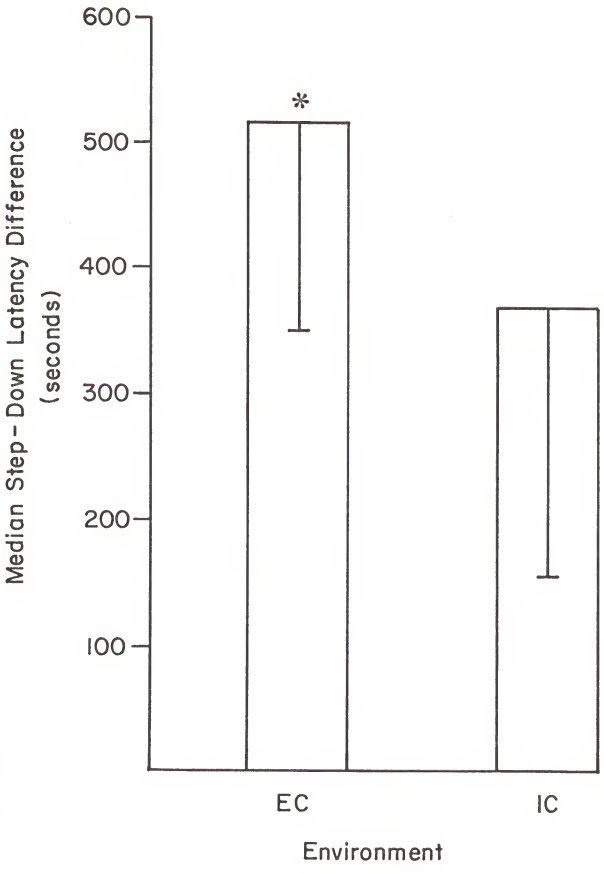
*$p \leq 0.05$

tend to lower that group's SDL difference scores. Nevertheless, SDL difference scores of the 4-month-old group were significantly higher than those of the older groups.

Analysis of the effect of environment on step-down passive avoidance. Statistical comparison of EC and IC animals indicated that environment did not significantly affect SDL difference scores in the 2-hr acquisition experiment χ^2 (1) = 0.24, $p > 0.62$. Median 2-hr SDL difference scores were 481 sec and 412 sec for the EC group (n = 25) and IC group (n = 2), respectively. There was no significant effect of environment on initial SDLs in this experiment χ^2 (1) = 0.41, $p > 0.52$. Median initial SDLs were 9 sec and 11 sec for the EC and IC groups, respectively.

In the 24-hr step-down retention experiment SDL difference scores of EC animals were significantly higher than those of IC animals χ^2 (1) = 4.19, $p < 0.04$. Figure 3 shows the median 24-hr SDL difference scores for the two groups. The median SDL difference score was 514 sec for the EC group (n = 44), compared to 368 sec for the IC group (n = 53). There was no significant difference between the two groups in their initial SDLs χ^2 (1) = 0.30, $p > 0.58$. Median initial SDLs were 6 sec and 8 sec for the EC and IC groups, respectively.

Figure 3. Comparison by environment of median SDL difference scores (+ interquartile deviation), using a 24-hr training-test interval. Medians for EC and IC groups are 514 and 368 sec, respectively. Asterisk indicates SDL difference scores of EC group are significantly higher than those of IC group.


600
500
400
300
200
100
*
Median Step-Down Latency Difference (seconds)
EC
IC
Environment

Receptor Binding

Determination of the effect of freezing

There was no statistically significant difference between fresh and previously frozen brain tissue in weight or protein values. Mean brain weights were 365 mg in the fresh group and 373 mg in the frozen group $\underline{t}$ (10) = 0.75, $p > 0.40$. In the first protein comparison (in which samples were taken immediately after homogenization), means were 0.543 mg/100 µl and 0.533 mg/100 µl for the fresh and frozen groups, respectively, $\underline{t}$ (10) = 0.61, $p > 0.40$. Mean protein values from the second comparison (in which homogenates derived from either fresh or previously frozen brain tissue were frozen, thawed, and resuspended) were 0.527 mg/100 µl in the fresh group and 0.524 mg/100 µl in the frozen group $\underline{t}$ (10) = 0.18, $p > 0.60$.

Binding of [^{3}H]QNB and [^{3}H]FNP was not significantly affected by prior freezing of brain tissue. Results of the first comparison (in which the binding assays were performed immediately after homogenization) indicated that mean binding of [^{3}H]QNB was 883 fmol/mg protein in the fresh group and 874 fmol/mg protein in the frozen group $\underline{t}$ (10) = 0.48, $p > 0.60$. Mean binding values of [^{3}H]FNP were 890 fmol/mg protein and 877 fmol/mg protein for the fresh and frozen groups, respectively, $\underline{t}$ (10) = 0.57, $p > 0.40$.

The second comparison (in which homogenates from fresh and previously frozen brains were frozen, thawed, and resuspended prior to assay) revealed a 3% decrease in [^{3}H]QNB

binding in the originally frozen samples, compared to the originally fresh samples, that approached statistical significance t (10) = 1.86, $p < 0.10$ (2-tailed). Means for binding of [^{3}H]QNB were 920 fmol/mg protein in the fresh group and 890 fmol/mg protein in the frozen group. Means for binding of [^{3}H]FNP were 906 fmol/mg protein and 895 fmol/mg protein for the fresh and frozen groups, respectively, t (10) = 0.55, $p > 0.40$.

In view of the negligible effect of prior freezing of brain tissue on binding of [^{3}H]QNB and [^{3}H]FNP, the procedure described in Chapter II was adopted for the receptor binding experiments in this investigation. Any minimal effect of prior freezing on binding values should have affected all samples equally and would not have influenced the results of different treatment comparisons.

Effect of age and environment on [^{3}H]QNB binding

Results of two-way ANOVA procedures for the effects of age and environment on [^{3}H]QNB binding in each brain region are described below.

Cerebral cortex. A two-way ANOVA procedure indicated no significant effect of age on [^{3}H]QNB binding in the cerebral cortex F (4, 61) = 1.37, $p > 0.25$. Mean binding values of [^{3}H]QNB in the 5 age groups are shown in Table 4. The effect of environment on [^{3}H]QNB binding was not significant F (2, 61) = 1.13, $p > 0.32$. Means for binding of [^{3}H]QNB in the SC, EC, and IC groups are shown in Table 5.

TABLE 4

EFFECT OF AGE ON REGIONAL BINDING OF [^{3}H]QNB IN THE C57BL/6 MOUSE

Region	Age									
	4 Months		8 Months		15 Months		20 Months		25 Months	
	[^{3}H]QNB* (fmol/mg protein)	n	[^{3}H]QNB* (fmol/mg protein)	n	[^{3}H]QNB* (fmol/mg protein)	n	[^{3}H]QNB* (fmol/mg protein)	n	[^{3}H]QNB* (fmol/mg protein)	n
Cerebral Cortex	1096 ± 20	(14)	1050 ± 48	(15)	1099 ± 32	(15)	1056 ± 30	(16)	973 ± 45	(16)
Striatum	1195 ± 54	(15)	1133 ± 47	(16)	1077 ± 34	(16)	1061 ± 39	(16)	1065 ± 59	(16)
Hippocampus	710 ± 41	(12)	803 ± 20	(12)	792 ± 18	(12)	763 ± 23	(11)	710 ± 22	(12)
Cerebellum	103 ± 8	(12)	92 ± 6	(11)	109 ± 5	(12)	89 ± 7	(12)	104 ± 4	(12)

*Mean ± S.E.M.

TABLE 5

EFFECT OF ENVIRONMENT ON REGIONAL [^{3}H]QNB BINDING IN THE C57/BL6 MOUSE

Region	Environment					
	SC		EC		IC	
	[^{3}H]QNB* (fmol/mg protein)	n	[^{3}H]QNB* (fmol/mg protein)	n	[^{3}H]QNB* (fmol/mg protein)	n
Cerebral Cortex	1102 ± 22	(20)	1052 ± 26	(28)	1020 ± 34	(28)
Striatum	1041 ± 39	(20)	1116 ± 37	(30)	1138 ± 34	(29)
Hippocampus	752 ± 24	(20)	777 ± 15	(19)	753 ± 23	(20)
Cerebellum	106 ± 4	(20)	101 ± 6	(20)	91 ± 5	(19)

*Mean ± S.E.M.

There was no significant interaction between the effects of age and environment on [^{3}H]QNB binding F (8, 61) = 0.99, $p > 0.44$.

Striatum. A two-way ANOVA procedure indicated that the effect of age on [^{3}H]QNB binding in the striatum was not statistically significant F (4, 64) = 1.61, $p > 0.18$. Mean binding values of [^{3}H]QNB in the 5 age groups are shown in Table 4. There was no significant effect of environment on [^{3}H]QNB binding F (2, 64) = 1.90, $p > 0.15$. Means for binding of [^{3}H]QNB in the SC, EC, and IC groups are shown in Table 5. There was no significant interaction between the effects of age and environment on [^{3}H]QNB binding F (8, 64) = 1.56, $p > 0.15$.

Hippocampus. The effect of age on [^{3}H]QNB binding in the hippocampus approached but did not reach statistical significance F (4, 44) = 2.14, $p > 0.09$. Mean binding values in the 5 age groups are shown in Table 4. There was no significant effect of environment on [^{3}H]QNB binding F (2, 44) = 0.43, $p > 0.65$. Means for binding of [^{3}H]QNB in the SC, EC, and IC groups are shown in Table 5. There was no significant interaction between the effects of age and environment on [^{3}H]QNB binding F (8, 44) = 0.64, $p > 0.74$.

Cerebellum. Results of a two-way ANOVA procedure indicated no significant effect of age on [^{3}H]QNB binding in the cerebellum F (4, 44) = 1.58, $p > 0.19$. Mean binding values of [^{3}H]QNB in the 5 age groups are shown in Table 4. The effect of environment on [^{3}H]QNB binding was not statistically

significant F (2, 44) = 2.23, $p > 0.11$. Means for binding of [^{3}H]QNB in the SC, EC, and IC groups are shown in Table 5. There was no significant interaction between the effects of age and environment on [^{3}H]QNB binding F (8, 44) = 0.24, $p > 0.98$.

Relationship between performance and [^{3}H]QNB binding

Cerebral cortex. A one-way ANOVA procedure comparing good performers and poor performers indicated that the difference in cortical [^{3}H]QNB binding values approached but did not reach statistical significance F (1, 54) = 3.33, $p > 0.07$. Table 6 shows mean binding values of [^{3}H]QNB in cerebral cortex of good and poor performers.

Striatum. There was no significant difference between good and poor performers in [^{3}H]QNB binding in the striatum F (1, 57) = 0.68, $p > 0.41$. Mean binding values of the two groups are shown in Table 6.

Hippocampus. As in the cerebral cortex, a comparison of good vs. poor performers in [^{3}H]QNB binding approached but did not reach statistical significance F (1, 37) = 3.35, $p > 0.07$. Table 6 shows mean binding values of [^{3}H]QNB in cerebral cortex of good and poor performers.

Cerebellum. There was no significant difference between good and poor performers in [^{3}H]QNB binding in the cerebellum F (1, 37) = 0.00, $p > 0.98$. Mean binding values of the two groups are shown in Table 6.

TABLE 6

COMPARISON OF REGIONAL [^{3}H]QNB BINDING IN ANIMALS DEMONSTRATING GOOD AND POOR PERFORMANCE ON PASSIVE AVOIDANCE

Region	Performance Level			
	Good		Poor	
	[^{3}H]QNB* (fmol/mg protein)	n	[^{3}H]QNB* (fmol/mg protein)	n
Cerebral Cortex	1074 ± 18	(28)	998 ± 38	(28)
Striatum	1148 ± 35	(29)	1107 ± 36	(30)
Hippocampus	788 ± 16	(20)	739 ± 21	(19)
Cerebellum	96 ± 5	(20)	96 ± 6	(19)

*Mean ± S.E.M.

Additional statistical comparisons of performance and [^{3}H]QNB binding

In view of one-way ANOVA results in cerebral cortex and hippocampus which approached statistical significance, two-way ANOVA procedures were performed to determine whether there was an interaction between age and performance in relation to [^{3}H]QNB binding. The main effects of age and performance on [^{3}H]QNB binding in each region were comparable to the results described above. Analysis of the interaction between age and performance revealed no significant interaction between the two variables and [^{3}H]QNB binding in any brain region examined. Probability values of F ratios for these interactions were 0.79, 0.96, 0.26, and 0.38 for cerebral cortex, striatum, hippocampus, and cerebellum, respectively.

To examine the relationship between [^{3}H]QNB binding values and performance of individual animals, Spearman rank correlation coefficients were calculated. Binding values of [^{3}H]QNB were correlated with difference scores on step-through and step-down passive avoidance. The results of these correlations are shown in Table 7. The only correlation that was statistically significant was between [^{3}H]QNB binding in the hippocampus and 24 hr difference scores on step-down passive avoidance ($\rho = 0.475$, $p < 0.03$).

TABLE 7

SPEARMAN RANK CORRELATION COEFFICIENTS BETWEEN [^{3}H]QNB BINDING AND PASSIVE AVOIDANCE RETENTION

Region	Step-Through 5 days	Step-Down 24 hr
Cerebral Cortex	$\rho = 0.124$	$\rho = 0.172$
	$p > 0.42$	$p > 0.34$
Striatum	$\rho = 0.112$	$\rho = -0.048$
	$p > 0.45$	$p > 0.79$
Hippocampus	$\rho = 0.022$	$\rho = 0.475$
	$p > 0.89$	$p < 0.03$
Cerebellum	$\rho = 0.055$	$\rho = 0.026$
	$p > 0.75$	$p > 0.59$

Autopsies

Results of post-mortem examinations revealed 1 skin tumor in a 20-month-old animal in the SC condition. No other gross abnormalities were observed in the sample of animals included for autopsy in Experiment 1.

Summary of Results in Experiment 1

Behavioral comparisons

Statistical analysis of the frequency of spontaneous alternation indicated no effect of age or environment on this behavior. The effect of age on acquisition of step-through passive avoidance was statistically significant, and was accounted for by a deficit in the 25-month-old group compared to the younger age groups. Five-day retention of step-through passive avoidance was also significantly affected by age. Performance of 15-, 20-, and 25-month-old animals was impaired relative to 4-month-old animals. Environment did not significantly affect acquisition or retention of step-through passive avoidance. The effect of age on acquisition of step-down passive avoidance was not statistically significant. Twenty-four hour retention of step-down passive avoidance was significantly affected by age. Performance of 15-, 20-, and 25-month-old animals was impaired relative to 4-month-old animals. Animals exposed to the EC environment performed significantly better than IC animals on 24-hr retention of the step-down task.

Receptor binding

A control experiment performed to determine the effects of prior freezing on binding of [^{3}H]QNB and [^{3}H]FNP revealed negligible effects on brain weight, protein values, and binding of [^{3}H]QNB and [^{3}H]FNP. The effects of age and environment on [^{3}H]QNB binding were not statistically significant in any of the brain regions examined. Interactions between the effects of age and environment on [^{3}H]QNB binding were not significant in any region. Analysis of [^{3}H]QNB binding in good vs. poor performers indicated differences in cerebral cortex and hippocampus which approached but did not reach statistical significance. Spearman rank correlation coefficients revealed a significant positive correlation only between 24-hr step-down passive avoidance performance and [^{3}H]QNB binding in the hippocampus.

Experiment 2

Effect of Scopolamine on Locomotor Activity

There was no significant difference in 1-hr activity counts between saline-injected controls and non-injected controls t (12) = 0.35, $p > 0.60$. The mean of activity counts in the saline-injected group was 956, compared to 1007 in the non-injected group. Because there was no significant difference between the 2 control groups, data from both groups were combined to form one larger control group.

The results of a two-way ANOVA procedure revealed a significant effect of drug treatment on activity counts

F (1, 20) = 6.40, $p < 0.02$. The mean of activity counts was 981 in the control group (n = 14) and 1427 in the scopolamine-treated group (n = 10). This comparison is shown in Figure 4A.

A comparison of 4- vs. 25-month-old animals indicated that the effect of age on activity was not statistically significant F (1, 20) = 1.48, $p > 0.23$. The mean of activity counts was 1239 in the 4-month-old group (n = 15) and 1046 in the 25-month-old group (n = 9). Figure 4B shows a comparison of mean activity counts in the two age groups.

The ANOVA procedure also indicated no significant interaction between the effects of drug treatment and age on activity F (1, 20) = 0.01, $p > 0.90$. Figure 4C shows a comparison of control vs. scopolamine-treated animals in each age group. The activity-stimulating effect of scopolamine was similar in each age group. Scopolamine administration increased activity relative to controls by approximately 45% in the 4-month-old group and 50% in the 25-month-old group.

Effect of Scopolamine on [^{3}H]QNB Binding

Hippocampus

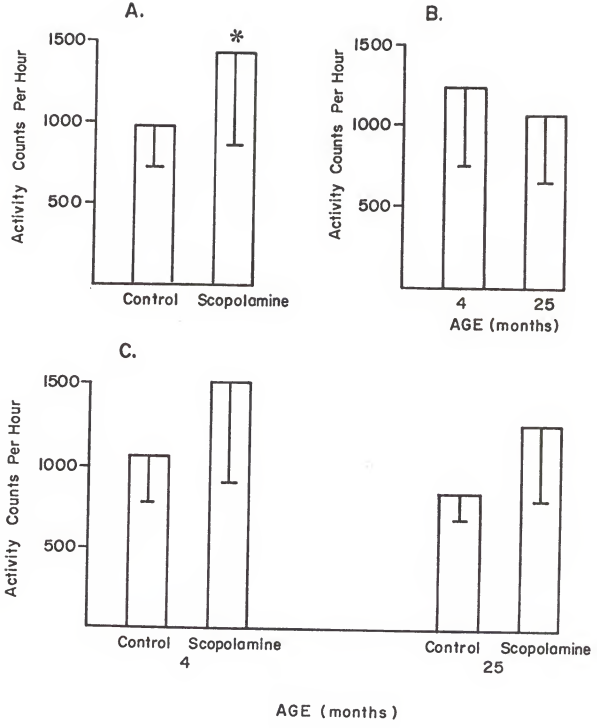
There was no significant difference between the saline-injected control group and the non-injected control group in binding of [^{3}H]QNB in the hippocampus t (12) = 0.44, $p > 0.60$. Mean binding of [^{3}H]QNB was 765 fmol/mg protein in the saline group, compared to 781 fmol/mg protein in the non-injected

Figure 4. Comparison of effects of drug treatment and age on locomotor activity.

A. Bar graph of mean total activity counts (± S.E.M.) in 1 hr, compared by drug treatment condition.

B. Bar graph of mean total activity counts (± S.E.M.) in 1 hr, compared by age.

C. Bar graph of mean total activity counts (± S.E.M.) in 1 hr, compared by drug treatment condition for each age group.


A.
1500
1000
500
Activity Counts Per Hour
*
Control
Scopolamine
B.
1500
1000
500
Activity Counts Per Hour
4
25
AGE (months)
C.
1500
1000
500
Activity Counts Per Hour
Control
Scopolamine
4
Control
Scopolamine
25
AGE (months)

control group. Therefore, data from the 2 control groups were combined for comparison to the scopolamine-treated groups.

Results of a two-way ANOVA procedure indicated no difference between scopolamine-treated animals and controls in binding of [^{3}H]QNB in the hippocampus F (1, 21) = 0.39, $p > 0.54$. The mean binding value was 789 fmol/mg protein in the scopolamine-treated group (n = 11) and 776 fmol/mg protein in the control group (n = 14).

The effect of age on [^{3}H]QNB binding in the hippocampus was also not significant in this experiment F (1, 21) = 0.44, $p > 0.51$. The mean binding value was 788 fmol/mg protein in the 4-month-old group (n = 15) and 772 fmol/mg protein in the 25-month-old group (n = 10).

There was no significant interaction between the effects of drug treatment and age on binding of [^{3}H]QNB in the hippocampus F (1, 21) = 0.10, $p > 0.76$. This result indicates that the effect of scopolamine on [^{3}H]QNB binding in the hippocampus did not vary as a function of age.

Cerebral cortex

There was no significant difference between the saline-injected control group and the non-injected control group in [^{3}H]QNB binding in the cerebral cortex t (7) = 0.38, $p > 0.60$. Mean binding was 974 fmol/mg protein in the saline group and 944 fmol/mg protein in the non-injected group. As described above, the data from the 2 control

groups were combined for comparison to the scopolamine-treated group.

A two-way ANOVA procedure revealed no significant effect of drug treatment on binding of [^{3}H]QNB in cerebral cortex F (1, 16) = 1.13, $p > 0.30$. Mean of binding was 957 fmol/mg protein in the control group (n = 9) and 903 fmol/mg protein in the scopolamine-treated group (n = 11).

The effect of age on binding of [^{3}H]QNB in cerebral cortex was statistically significant F (1, 16) = 5.02, $p < 0.04$. Mean binding values were 974 fmol/mg protein for the 4-month-old group and 842 fmol/mg protein for the 25-month-old group.

There was no significant interaction between the effect of drug treatment and age F (1, 16) = 0.32, $p > 0.57$. This result indicates that the effect of scopolamine on [^{3}H]QNB binding in cerebral cortex did not differ as a function of age.

Autopsies

Results of post-mortem examinations revealed no gross abnormalities in any of the animals used in this experiment.

Experiment 3

Statistically significant age-related differences in binding of [^{3}H]QNB and [^{3}H]FNP were observed in several

brain regions in Experiment 3. Because of the small amount of tissue available, Scatchard analyses were performed on homogenates pooled from the 8 animals in each age group. This quantity of tissue was sufficient for determination of 4 points on the Scatchard plots. Since pooling of tissue was necessary and only 4 points were examined, no statistical analysis was possible, and the results of the Scatchard analyses can only be considered suggestive. Two representative Scatchard plots are included in this manuscript.

[^{3}H]QNB Binding

Cerebral cortex

The effect of age on [^{3}H]QNB binding in cerebral cortex was statistically significant t (14) = 12.23, $p < 0.0005$. Table 8 shows mean [^{3}H]QNB and [^{3}H]FNP binding results in the 2 age groups for all 4 brain regions. Binding of [^{3}H]QNB in cerebral cortex of the 29-month-old group was decreased by 20% compared to the 4-month-old group. Scatchard analysis indicated a decrease in B_{max} (maximal specific binding) and a 17% decrease in the K_d value of the aged animals. This suggests a decrease in receptor density and a possible increase in receptor affinity in the aged group.

Striatum

A statistically significant age-related decrease in [^{3}H]QNB binding was observed in the striatum t (14) = 16.53, $p < 0.0005$. Means for the 4- and 24-month-old groups are

TABLE 8

AGE COMPARISON OF REGIONAL BINDING OF [^{3}H]QNB AND [^{3}H]FNP IN THE C57BL/6J MOUSE

Region	[^{3}H]QNB Binding* (fmol/mg protein)		[^{3}H]FNP Binding* (fmol/mg protein)	
	Age		Age	
	4 Months	29 Months	4 Months	29 Months
Cerebral Cortex	1185 ± 14	950** ± 13	1186 ± 23	1039** ± 22
Striatum	1473 ± 20	1067** ± 14	467 ± 16	426 ± 18
Hippocampus	939 ± 37	840** ± 23	899 ± 16	781** ± 32
Cerebellum	121 ± 7	115 ± 5	538 ± 7	459** ± 9

*Mean ± S.E.M. of 8 animals in each age group.

**Significantly different from 4-month-old group ($p < 0.05$).

shown in Table 8, which indicates a 28% decrease in striatal [^{3}H]QNB binding in the aged group. Scatchard analysis, shown in Figure 5, indicated a decrease in B_{max} in the aged group and also a 35% decease in K_d. As in the cerebral cortex, these data suggest a decrease in receptor density and a possible increase in receptor affinity.

Hippocampus

The effect of age on [^{3}H]QNB binding in the hippocampus was also statistically significant t (14) = 10.5, $p < 0.25$. A comparison of the 2 age groups is shown in Table 8. Binding was decreased by 11% in the 29-month-old group compared to the 4-month-old group. Scatchard analysis suggested no differences in affinity for [^{3}H]QNB between young and aged animals.

Cerebellum

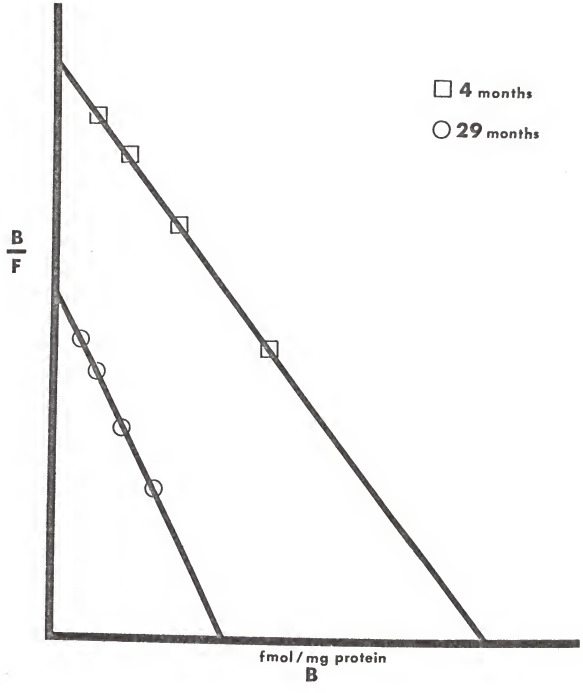
There was no statistically significant effect of age on [^{3}H]QNB binding in the cerebellum t (14) = 0.38, $p > 0.30$. Mean binding of [^{3}H]QNB in the 2 age groups is shown in Table 8.

[^{3}H]FNP Binding

Cerebral cortex

The effect of age on [^{3}H]FNP binding in the cerebral cortex was statistically significant t (14) = 4.60, $p < 0.0005$. A comparison of mean cortical binding of [^{3}H]FNP

Figure 5. Scatchard plot age comparison of [^{3}H]QNB binding in the striatum. Increased B_{max} is represented by displacement to the right on the X-axis. Increased affinity is represented by an increase in slope.


□ 4 months
○ 29 months
B/F
fmol / mg protein
B

in the 4- and 29-month-old groups is shown in Table 8. Binding was decreased by 12% in the aged group. Results of a Scatchard plot indicated a decrease in B_{max} and a 14% decrease in K_d in the aged animals, suggesting a decrease in receptor density and a possible increase in receptor affinity.

Striatum

The effect of age on [^{3}H]FNP binding in striatum did not reach statistical significance $\underline{t}$ (14) = 1.69, $p > 0.05$. Mean binding of [^{3}H]FNP in the 2 age groups is shown in Table 8.

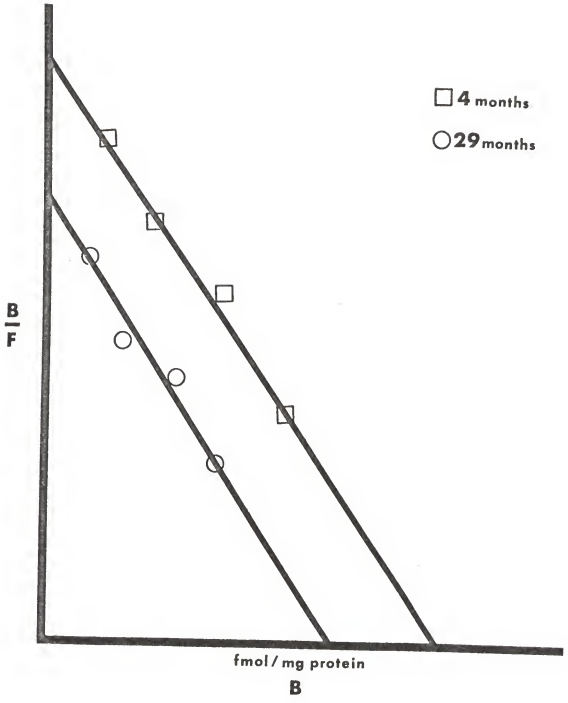
Hippocampus

A statistically significant effect of age on [^{3}H]FNP binding was observed in the hippocampus $\underline{t}$ (13) = 3.16, $p < 0.005$. Table 8 shows a comparison of mean hippocampal binding of [^{3}H]FNP in the 4- and 29-month-old age groups. A 13% decrease was found in the aged group. Scatchard analysis, shown in Figure 6, indicated an age-related decrease in B_{max} but no change in the K_d value. This suggests an age-related decrease in receptor density with no alteration in receptor affinity.

Cerebellum

The effect of age on [^{3}H]FNP binding in the cerebellum was also statistically significant $\underline{t}$ (14) = 7.07, $p < 0.0005$.

Figure 6. Scatchard plot age comparison of [^{3}H]FNP binding in the hippocampus. Increased Bmax is represented by displacement to the right on the X-axis. Increased affinity is represented by an increase in slope.


□ 4 months
○ 29 months
B/F
fmol / mg protein
B

A comparison of [^{3}H]FNP binding in the 2 age groups is shown in Table 8. Binding in the 29-month-old group was decreased by 15% in comparison to the 4-month-old group. Scatchard analysis indicated a decrease in B_{max} and also a 12% increase in K_d in the aged animals. This suggests a decrease in receptor density in the cerebellum and a possible decrease in receptor affinity.

Autopsies

The results of the autopsies indicated no abnormalities in the 8 young animals examined. Three of the aged mice appeared to have pituitary tumors. A pleural exudate was also observed in 1 of 3 mice with probable tumors. Atrophy of gonadal tissue was observed in all of the aged mice.

In view of the relatively high incidence of pathology in the aged group, binding values in the 3 abnormal animals were compared to binding values in the 5 healthy-appearing aged animals for each region. The results of these comparisons are shown in Table 9. There was no significant difference between these 2 groups in [^{3}H]QNB binding or [^{3}H]FNP binding in any of the brain regions examined.

TABLE 9

COMPARISON OF REGIONAL BINDING OF [^{3}H]QNB AND [^{3}H]FNP IN AGED C57BL/6J MICE WITH NORMAL AND ABNORMAL FINDINGS AT AUTOPSY

Region	[^{3}H]QNB Binding* (fmol/mg protein)		[^{3}H]FNP Binding* (fmol/mg protein)	
	Normal	Abnormal	Normal	Abnormal
Cerebral Cortex	957 ± 20	940 ± 10	1024 ± 25	1063 ± 44
	$t(6) = 0.60$ $p > 0.20$		$t(6) = 0.85$ $p > 0.20$	
Striatum	1056 ± 14	1085 ± 32	433 ± 29	453 ± 50
	$t(6) = 0.98$ $p > 0.10$		$t(6) = 0.38$ $p > 0.30$	
Hippocampus	842 ± 36	838 ± 25	767 ± 44	806 ± 47
	$t(6) = 0.06$ $p > 0.30$		$t(6) = 0.57$ $p > 0.20$	
Cerebellum	114 ± 8	117 ± 5	461 ± 11	456 ± 4
	$t(6) = 0.25$ $p > 0.30$		$t(6) = 0.30$ $p > 0.30$	

*Mean ± S.E.M. for each group.

CHAPTER IV

DISCUSSION

The results of this investigation, on the whole, do not support the cholinergic hypothesis of age-related memory loss. In Experiment 1 significant retention deficits on passive avoidance tasks were observed in 15-, 20-, and 25-month-old male C57BL/6 mice compared to 4- and 8-month-old mice. A comparison of [^{3}H]QNB binding in the same animals from these 5 age groups revealed no significant effect of age on [^{3}H]QNB binding in the cerebral cortex, striatum, hippocampus, or cerebellum.

This dissociation between the temporal onset of behavioral deficits and alterations in [^{3}H]QNB binding is in agreement with data reported by Strong et al. (1980). These investigators examined 24-hr retention of step-through passive avoidance and [^{3}H]QNB binding in cerebral cortex, striatum, and the hippocampus of 6-, 12-, and 30-month-old male C57BL/6J mice. Significant age-related performance deficits and an age-related decrease in [^{3}H]QNB binding in cerebral cortex and the striatum were reported. However, performance deficits were apparent in the 12- and 30-month-old groups compared to the 6-month-old group, while the decrease in [^{3}H]QNB binding was observed only in the

30-month-old group. Experiment 1 (using a 5-day training-test interval) indicated that the lack of association Strong et al. found in 12-month-old animals extends to include 15-, 20-, and 25-month-old animals. Additionally, the present investigation revealed similar results in regard to performance on a step-down passive avoidance task.

These results should not be interpreted to suggest that the cholinergic system is not related to memory function. Pharmacological manipulations of the cholinergic system have been shown to alter performance in both human and animal studies (e.g., Deutsch, 1973; Drachman & Leavitt, 1974). However, the finding that blockade of cholinergic receptors impairs retention in young animals (or humans) does not imply that all animals (or humans) with impaired retention must have altered cholinergic function. A wide variety of pharmacological agents affecting many different neurotransmitter systems have been shown to disrupt memory proces-ing (see Hunter, Zornetzer, Jarvik & McGaugh, 1977, and Zornetzer, 1978, for reviews). There is good evidence that the cholinergic system is involved in memory processing, but there is little reason to suspect that it is the only neurotransmitter system important in memory function.

Data in Experiment 1 suggestive of a relationship between [^{3}H]QNB binding and performance were obtained in the comparison of binding values in good vs. poor performers. Small differences (6-10%) in [^{3}H]QNB binding were observed

in cerebral cortex and hippocampus (binding in poor performers was lower than that in good performers) that approached statistical significance ($p < 0.08$). However, the effect of age on [^{3}H]QNB binding was not significant in this experiment, and there was no significant interaction between age and performance effects on [^{3}H]QNB binding in any region. Therefore, it is unlikely that this small difference, distributed across all age groups, could account for the retention deficits observed in the older age groups. A significant correlation ($\rho = 0.475$) was found between 24-hr retention of step-down passive avoidance and [^{3}H]QNB binding in the hippocampus. This finding indicates an association between hippocampal [^{3}H]QNB binding and performance on the step-down task (there was no correlation with step-through performance), but does not suggest that age-related deficits are caused by differences in [^{3}H]QNB binding. Since [^{3}H]QNB binding in the hippocampus did not decrease with aging, this is probably a moot point. A presumed behavioral indication of hippocampal function, spontaneous alternation, was also not significantly affected by age.

In regard to the nature of the age-related passive avoidance deficits observed in Experiment 1, one of the objectives of this investigation was to distinguish between acquisition deficits and retention deficits by using 2 different training-test intervals. On the step-through task, a significant 5-day retention deficit was found in 15-, 20-,

and 25-month-old animals, and a 2-hr deficit was also found in the 25-month-old group, indicating a deficit in acquisition. Therefore, the 5-day effect in the 25-month-old group may be partly, or entirely, a result of poor acquisition. It is interesting that this effect was not observed in the 15- and 20-month-old groups, which exhibited pronounced deficits at 5 days, but no deficit at 2 hr. These data indicate a specific deficit in retention in these animals. The trend toward better 5-day performance in 25-month-old animals compared to 20-month-old animals ($p < 0.07$) should also be mentioned here. There is no intuitively obvious explanation for this difference (assuming it is a real difference), but factors such as survival of a selected population of animals (the death rate remains very low at 20 months) or differences between breeders could have contributed to this effect.

Significant age-related deficits on 24-hr retention of step-down passive avoidance were found in the 15-, 20-, and 25-month-old groups. On this task there was no significant effect of age on 2-hr acquisition. This finding suggests that the 24-hr deficit was an actual retention deficit and not the result of impaired acquisition. However, it should be noted that 2-hr performance on this task was poorer and more variable than that on the 2-hr step-through task. This variability and weaker initial training could conceivably have obscured an aging effect on acquisition.

These data do not permit a clear cut characterization of the age-related deficits on passive avoidance tasks as either acquisition on retention deficits. In view of previous reports of performance deficits in rodents after short training-test intervals (Brizzee & Ordy, 1979; MaNamara et al., 1977), it appears that impaired acquisition is at least one factor in age-related performance deficits. The importance of including short and long intervals in studies of this sort is reemphasized by the present findings.

Environmental enrichment in Experiment 1 significantly improved retention in mice on 24-hr step-down passive avoidance compared to mice in the impoverished condition. This finding is in agreement with data by Gardner et al. (1975) who reported a deficit on 24-hr step-down performance in impoverished rats compared to enriched rats. Since SC animals were not tested in the present investigation, it cannot be determined whether the effect was the result of facilitation in the EC animals, impairment in the IC animals, or both, relative to animals reared under standard housing conditions.

The effect of environment on step-down performance was statistically significant in an overall comparison, in which animals in all age groups were considered together. Comparisons within individual age groups were not significant. This indicates that a large sample size was required for the effect to become apparent and also that no

particular age group was obviously more affected by the manipulation than the others.

Performance on step-through passive avoidance was not affected by environment in this experiment. Previous reports in the literature have shown facilitation, impairment, and no difference, in performance on the step-through task, in rodents exposed to enriched environments, compared to animals exposed to impoverished environments. It is possible, of course, that a longer duration of differential housing might have affected step-through performance in this experiment.

In regard to amelioration of age-related memory deficits through environmental manipulations, the step-down results can only be considered suggestive, since significant effects in individual age groups were not observed. Nevertheless, it is clear that performance of animals, living in a socially- and perceptually-enriched environment for 1 month prior to training, was superior on at least 1 task to that of animals housed in isolation.

No significant effect of environment on [^{3}H]QNB binding was observed in any of the brain regions examined. This suggests that the behavioral effect of environment was not mediated by an effect on [^{3}H]QNB binding.

In Experiment 2 administration of a high dose of scopolamine to 4- and 25-month-old mice was shown to have a significant locomotor-stimulating effect. Aged mice were neither less nor more sensitive to the drug than young mice

in regard to this behavioral effect. The proportional increase in activity was approximately equal in both age groups. This could be interpreted as indirect evidence for the functional integrity of at least part of the cholinergic system in 25-month-old mice.

However, the major purpose of Experiment 2 was to investigate the effect of chronic scopolamine treatment on [^{3}H]QNB binding. The results did not replicate the findings by Ben Barak and Dudai (1980) in the rat. Seven days of scopolamine treatment had no effect on [^{3}H]QNB binding in the hippocampus of the C57BL/6 mouse, even though a very high dose was used in the present experiment. A pilot experiment, employing the same dose used by Ben-Barak and Dudai, also revealed no effect on [^{3}H]QNB binding.

Species differences in sensitivity to and metabolism of the drug may account for the discrepant results obtained. Rodents vary considerably in their ability to tolerate large doses of scopolamine, apparently due in part to the presence or absence of an enzyme, atropinesterase, in the blood and liver (Goodman & Gilman, 1980). A sedative effect of scopolamine has been reported in the mouse after administration of a 450 mg/kg dose (Tui & Debruille, 1945). In the rat, this sedative effect occurs with a dose of 13 mg/kg (Gruhzit & Dox, 1937). It is possible that in the mouse a larger dose, increased frequency, and/or longer duration of administration would result in an increase in [^{3}H]QNB binding.

Since no effect of the drug was found on [^{3}H]QNB binding in the hippocampus, it is not surprising that no effect was observed in cerebral cortex. The possibility of an aging effect on the reported compensatory response of the cholinergic system could not be evaluated, as scopolamine did not alter [^{3}H]QNB binding in either young or aged animals.

The comparison of young vs. aged animals on [^{3}H]QNB binding in cerebral cortex was statistically significant in Experiment 2. This finding does not actually conflict with the results of Experiment 1. Although in the first experiment the overall effect of age on [^{3}H]QNB binding in cerebral cortex was not significant, a Duncan's multiple range test indicated a significant 11% decrease in binding in the 25-month-old group compared to the 4-month-old group ($p < 0.05$). This test revealed a similar significant difference between the 25- and 15-month-old groups (binding in the 25-month-old group was 11% lower than binding in the 15-month-old group). No other individual comparisons in Experiment 1 indicated age-related decreases in any brain region. Taken together, these data indicate a relatively small (11-13%) decrease in cortical [^{3}H]QNB binding in 25-month-old mice compared to 4-month-old mice. No difference between 4- and 15- or 20-month-old animals was apparent in [^{3}H]QNB binding, even though at these ages animals exhibit pronounced behavioral deficits.

In Experiment 3, statistically significant differences between 4- and 29-month-old mice were observed in binding of [^{3}H]QNB and [^{3}H]FNP in several brain regions. The magnitude of the age-related decrease in [^{3}H]QNB binding was 20% in cerebral cortex, 28% in striatum, and 11% in the hippocampus. These data are in general agreement with those reported by Strong et al. (1980), who found comparable decreases in 30- vs. 6-month-old mice. The results of the Scatchard analyses in Experiment 3 must be regarded as highly speculative. Nevertheless, Strong et al. (1980) reported a significant age-related increase in affinity for [^{3}H]QNB in the rat striatum (in addition to a decrease in B_{max}), an effect also suggested by the present investigation. The results of Experiment 3 indicated that the largest age-related decrease in [^{3}H]QNB binding occurred in the striatum. This finding is in accordance with most of the other regional studies in the animal literature. Significant decreases in cerebral cortex and the hippocampus suggest that these structures are also affected by aging, but to a lesser extent.

It should be noted that Strong et al. (1980) used male C57BL/6J mice as subjects, while female C57BL/6J mice were used in Experiment 3. Comparable aging effects were observed. Therefore, it is unlikely that the obvious age differences in [^{3}H]QNB binding found in Experiment 3 (in comparison to Experiment 1) were the result of a gender difference between the two studies. On the whole, it appears

that [^{3}H]QNB binding remains stable for most of the life-span of the C57BL/6 mouse and declines relatively suddenly as animals approach 30 months of age.

The effect of age on [^{3}H]FNP binding had not been reported in the literature as of this writing. In the present investigation, significant 12-15% decreases were observed in the cerebral cortex, hippocampus, and cerebellum of 29-month-old mice compared to 4-month-old mice. This finding could be related to reports of increased anxiety and fear in aged organisms.

Comparison of apparently healthy 29-month-old animals and animals with pathological findings at autopsy revealed no difference between the 2 groups in binding of either [^{3}H]QNB or [^{3}H]FNP in any brain region. This suggests that pathological conditions associated with aging are not necessarily responsible for the aging effects observed. It should be reiterated that Freund (1980) reported no effect of severe weight loss and nonspecific illness on binding of [^{3}H]QNB in the C57BL/6J mouse brain. Nevertheless, direct effects of aging on the cholinergic system cannot, at present, be dissociated from indirect effects mediated via other age-related changes.

In conclusion, both age-related alterations in behavior and age-related regional decreases in [^{3}H]QNB binding were observed in these experiments. However, there appeared to be little temporal association between these 2 concomitants of the aging process. The data collected do not support

the cholinergic hypothesis of age-related memory loss. Regional binding of [^{3}H]QNB is only one index of cholinergic function, and it is possible that other indices might correlate more closely with age-related memory loss. Decreases in activity of CAT with aging do not coincide with the onset of memory dysfunction. Therefore, proponents of the cholinergic hypothesis in its purest form must invoke presently obscure changes in the system to account for the early appearance of behavioral deficits.

If age-related alterations in the cholinergic system are not responsible for the memory loss associated with aging, what are the neurobiological substrates of this phenomenon? The present investigation examined only [^{3}H]QNB binding in relation to behavior. A myriad of neurochemical, neuroanatomical, and neurophysiological changes have been reported in aged organisms, including changes in other neurotransmitter systems (particularly noradrenergic and dopaminergic systems) implicated in memory function. It is conceivable that alterations in putative endogenous benzodiazepine systems could play a role in performance deficits, since fear is an important component of avoidance training paridigms. Combined investigation of behavioral changes and alterations in other neurobiological systems may reveal aspects of brain function more closely linked to age-related memory loss.

REFERENCES

Arenberg, D. (1978). Differences and changes with age in the Benton Visual Retention Test. Journal of Gerontology, 33, 534-540.

Bartus, R.T. (1979). Physostigmine and recent memory: Effects in young and aged nonhuman primates. Science, 206, 1087-1089.

Bartus, R.T., Dean, R.L., III, and Fleming, D.L. (1979). Aging in the rhesus monkey: Effects on visual discrimination learning and reversal learning. Journal of Gerontology, 34, 209-219.

Bartus, R.T., Dean, R.L., Goas, J.A., and Lippa, A.S. (1980). Age-related changes in passive avoidance retention: Modulation with dietary choline. Science, 209, 301-303.

Ben-Barak, J., and Dudai, Y. (1980). Scopolamine induces an increase in muscarinic receptor level in rat hippocampus. Brain Research, 193, 309-313.

Bennett, E.L., Diamond, M.C., Krech, D., and Rosenzweig, M.R. (1964). Chemical and anatomical plasticity of brain. Science, 146, 610-619.

Bennett, E.L., Rosenzweig, M.R., Diamond, M.C., Morimoto, H., and Herbert, M. (1974). Effects of successive environments on brain measures. Physiology and Behavior, 12, 621-631.

Bennett, J.P., Jr. (1978). Methods in binding studies. In H.I. Yamamura, S.J. Enna, and M.J. Kuhar (Eds.), Neurotransmitter Receptor Binding, pp. 57-90. New York: Raven Press.

Bird, E.D., and Iversen, L.L. (1974). Huntingtons chorea: Post-mortem measurement of glutamic acid decarboxylase, choline acetyltransferase and dopamine in basal ganglia. Brain, 97, 457-472.

Botwinick, J. (1973). Aging and Behavior. New York: Springer.

Botwinick, J., and Storandt, M. (1974). Memory, Related Functions, and Age. Springfield, Ill.: Charles C. Thomas.

Bousfield, A.K., and Bousfield, W.A. (1966). Measurement of clustering and of sequential constancies in repeated free recall. Psychological Reports, 19, 935-942.

Brizzee, K.R., and Ordy, J.M. (1979). Age pigments, cell loss and hippocampal function. Mechanisms of Ageing and Development, 9, 143-162.

Buschke, H. (1974). Two stages of learning by children and adults. Bulletin of the Psychonomic Society, 2, 392-394.

Canestrari, R.E. (1963). Paced and self-paced learning in young and elderly adults. Journal of Gerontology, 18, 165-168.

Carlton, P.L. (1968). Cholinergic mechanisms in the control of behavior. In D.H. Efron (Ed.), Psychopharmacology - A Review of Progress 1957-1967, pp. 125-135. Washington, D.C.: U.S. Govt. Printing Office.

Craik, F.I.M. (1977). Age differences in human memory. In J.E. Birren and K.W. Schaie (Eds.), Handbook of the Psychology of Aging, pp. 384-420. New York: Van Nostrand Reinhold.

Craik, F.I.M., and Masani, P.A. (1969). Age and intelligence differences in coding and retrieval of word lists. British Journal of Psychology, 60, 315-319.

Craik, F.I.M., and Tulving, E. (1975). Depth of processing and the retention of words in episodic memory. Journal of Experimental Psychology: General, 104, 268-294.

Crowder, R.G. (1976). Principles of Learning and Memory. Hillsdale, N.J.: Erlbaum.

Cummins, R.A., Walsh, R.N., Budtz-Olsen, O.E., Konstantinos, T., and Horsfall, C.R. (1973). Environmentally-induced changes in the brains of elderly rats. Nature, 243, 516-518.

Cutting, W.C. (1964). Handbook of Pharmacology . . . The actions and uses of drugs. New York: Appleton-Century-Crofts.

Davies, P., and Verth, A.H. (1977). Regional distribution of muscarinic acetylcholine receptor in normal and Alzheimer's-type dementia brains. Brain Research, 138, 385-392.

Davis, K.L., and Yamamura, H.I. (1978). Cholinergic underactivity in human memory disorders. Life Sciences, 23, 1729-1734.

Dean, R.L., Goas, J.A., Lippa, A.S., Bartus, R.T., Pelham, R., and Marquis, J.K. (1979). Changes in behavior and receptor binding in aged vs. young mice. Society for Neuroscience Abstracts, 5, 5.

Deutsch, J.A. (1973). The cholinergic synapse and the site of memory. In J.A. Deutsch (Ed.), The Physiological Basis of Memory, pp. 59-76. New York: Academic Press.

Doty, B.A. (1966). Age and avoidance conditioning in rats. Journal of Gerontology, 21, 287-290. (a).

Doty, B.A. (1966). Age differences in avoidance conditioning as a function of distribution of trials and task difficulty. Journal of Genetic Psychology, 109, 249-254. (b).

Doty, B.A. (1968). Effects of handling on learning of young and aged rats. Journal of Gerontology, 23, 142-144.

Drachman, D.A., and Leavitt, J.L. (1974). Human memory and the cholinergic system. A relationship to aging? Archives of Neurology, 30, 113-121.

Erber, J.T. (1974). Age differences in recognition memory. Journal of Gerontology, 29, 177-181.

Erber, J.T. (1978). Age differences in a controlled-lag recognition memory task. Experimental Aging Research, 4, 195-205.

Eysenck, M.W. (1974). Age differences in incidental learning. Developmental Psychology, 10, 936-941.

Finch, C.E. (1977). Neuroendocrine and autonomic aspects of aging. In C.E. Finch and L. Hayflick (Eds.), Handbook of the Biology of Aging, pp. 262-280. New York: Van Nostrand Reinhold.

Freeman, B.J., and Ray, O.S. (1972). Strain, sex, and environment effects on appetitively and aversively motivated learning tasks. Developmental Psychobiology, 5, 101-109.

Freund, G. (1980). Cholinergic receptor loss in brains of aging mice. Life Sciences, 26, 371-375.

Freund, G., and Walker, D.W. (1971). The effects of aging on acquisition and retention of shuttle box avoidance in mice. Life Sciences, 10, 1343-1349.

Gardner, E.B., Boitano, J.J., Mancino, N.S., D'Amico, D.P., and Gardner, E.L. (1975). Environmental enrichment and deprivation: Effects on learning, memory and exploration. Physiology and Behavior, 14, 321-327.

Gilbert, J.G. (1941). Memory loss in senescence. Journal of Abnormal and Social Psychology, 36, 73-86.

Gilman, A.G., Goodman, L.S., and Gilman, A. (1980). Goodman and Gilman's the Pharmacological Basis of Therapeutics. New York: Macmillan.

Gold, P.E., and McGaugh, J.L. (1975). Changes in learning and memory during aging. In J.M. Ordy and K.R. Brizzee (Eds.), Neurobiology of Aging. New York: Plenum Press.

Gold, P.E., McGaugh, J.L., Hankins, L.L., Rose, R.P., and Vasquez, B.J. (1981). Physiology and Behavior, in press.

Goodrick, C.L. (1968). Learning, retention, and extinction of a complex maze habit for mature-young and senescent Wistar albino rats. Journal of Gerontology, 23, 298-304.

Goodrick, C.L. (1972). Learning by mature-young and aged Wistar albino rats as a function of test complexity. Journal of Gerontology, 27, 353-357.

Goodrick, C.L. (1973). Maze learning of mature-young and aged rats as a function of distribution of practice. Journal of Experimental Psychology, 98, 344-349.

Goodrick, C.L. (1975). Behavioral rigidity as a mechanism for facilitation of problem solving for aged rats. Journal of Gerontology, 30, 181-184.

Greenberg, L.H., and Weiss, B. (1978). β-adrenergic receptors in rat brain: Reduced number and capacity of pineal gland to develop supersensitivity. Science, 201, 61-63.

Greenberg, L.H., and Weiss, B. (1979). Ability of aged rats to alter beta adrenergic receptors of brain in response to repeated administration of reserpine and desmethylimipramine. Journal of Pharmacology and Experimental Therapeutics, 211, 309-316.

Greenough, W.T., Fulcher, J.K., Yuwiler, A., and Geller, E. (1970). Enriched rearing and chronic electroshock: Effects on brain and behavior in mice. Physiology and Behavior, 5, 371-373.

Gruhzit, O.M., and Dox, A.W. (1937). A pharmacologic study of certain thiobarbiturates. Journal of Pharmacology and Experimental Therapeutics, 60, 125-142.

Harwood, E., and Naylor, G.F.K. (1969). Recall and recognition in elderly and young subjects. Australian Journal of Psychology, 21, 251-257.

Hicks, P., Rolsten, C., Hsu, L., Schoolar, J., and Samorajski, T. (1979). Brain uptake index for choline in aged rats. Society for Neuroscience Abstracts, 5, 6.

Hollander, J., and Barrows, C.H. (1968). Enzymatic studies in senescent rodent brain. Journal of Gerontology, 23, 174-179.

Hulicka, I.M., and Grossman, J.L. (1967). Age group comparisons for the use of mediators in paired-associate learning. Journal of Gerontology, 22, 46-51.

Hulicka, I.M., Sterns, H., and Grossman, J. (1967). Age group comparisons of paired-associate learning as a function of paced and self-paced association and response times. Journal of Gerontology, 22, 274-280.

Hultsch, D.F. (1969). Adult age differences in the organization of free recall. Developmental Psychology, 1, 673-678.

Hultsch, D.F. (1971). Adult age differences in free classification and free recall. Developmental Psychology, 4, 338-342.

Hultsch, D.F. (1974). Learning to learn in adulthood. Journal of Gerontology, 29, 302-308.

Hunter, B., Zornetzer, S.F., Jarvik, M.E., and McGaugh, J.L. (1977). Modulation of learning and memory: effects of drugs influencing neurotransmitters. In L.L. Iversen, S.D. Iversen, and S.H. Snyder (Eds.), Handbook of Psychopharmacology, Vol. 8: Drugs, Neurotransmitters, and Behavior, pp. 531-577. New York: Plenum Press.

James, T.C., and Kanungo, M.S. (1976). Alterations in atropine sites of the brain of rats as a function of age. Biochemical and Biophysical Research Communications, 72, 170-175.

Johnson, L.K. (1973). Changes in Memory as a Function of Age. Doctoral dissertation, University of Southern California.

Kubanis, P., Gobbel, G., and Zornetzer, S.F. (1981). Age-related memory deficits in Swiss mice. Behavioral and Neural Biology, in press.

Kubanis, P., and Zornetzer, S.F. (1981). Age-related behavioral and neurobiological changes: A review with an emphasis on memory. Behavioral and Neural Biology, 31, 115-172.

Kuhar, M.J. (1976). The anatomy of cholinergic neurons. In A.M. Goldberg and I. Hanin (Eds.), Biology of Cholinergic Function, pp. 3-27. New York: Raven Press.

Labouvie-Vief, G., Hoyer, W.J., Baltes, M.M., and Baltes, P.B. (1974). Operant analysis of intellectual behavior in old age. Human Development, 17, 259-272.

Laurence, M.W. (1966). Age differences in performance and subjective organization in the free recall of pictorial material. Canadian Journal of Psychology, 20, 388-399.

Laurence, M.W. (1967). Memory loss with age: A test of two strategies for its retardation. Psychonomics Science, 9, 209-210.

Lippa, A.S., Critchett, D.J., Bartus, R.T., Harrington, W., and Pelham, R.W. (1979). Electrophysiological and biochemical evidence for age-related alterations in hippocampal cholinergic functioning. Society for Neuroscience Abstracts, 5, 8.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. Journal of Biological Chemistry, 193, 265-275.

Martin, I.L. (1980). Endogenous ligands for benzodiazepine receptors. Trends in Neurosciences, 3, 299-301.

McGeer, E.G., Fibiger, H.C., McGeer, P.L., and Wickson, V. (1971). Aging and brain enzymes. Experimental Gerontology, 6, 391-396.

McGeer, E.G., and McGeer, P.L. (1975). Age changes in the human for some enzymes associated with metabolism of catecholamines, GABA, and acetylcholine. In J.J. Ordy and K.R. Brizzee (Eds.), Neurobiology of Aging, pp. 287-305. New York: Plenum Press.

McNamara, M.C., Benignus, V.A., Benignus, G., and Miller, A.T. (1977). Active and passive avoidance in rats as a function of age. Experimental Aging Research, 3, 3-16.

Medin, D.L., O'Neil, P., Smeltz, E., and Davis, R.T. (1973). Age differences in retention of concurrent discrimination problems in monkeys. Journal of Gerontology, 28, 63-67.

Meek, J.L., Bertilsson, L., Cheney, D.L., Zsilla, G., and Costa, E. (1977). Aging-induced changes in acetylcholine and serotonin content of discrete brain nuclei. Journal of Gerontology, 32, 129-131.

Mohs, R.C., Davis, K.L., Tinklenberg, J.R., Hollister, L.E., Yesavage, J.A., and Kopell, B.S. (1979). Choline chloride treatment of memory deficits in the elderly. American Journal of Psychiatry, 136, 1275-1277.

Monge, R., and Hultsch, D. (1971). Paired associate learning as a function of adult age and length of anticipation and inspection intervals. Journal of Gerontology, 26, 157-162.

Morin, A.M., and Wasterlain, C.G. (1980). Aging and rat brain muscarinic receptors as measured by quinuclidinyl benzilate binding. Neurochemical Research, 5, 301-308.

Moudgil, V.K., and Kanungo, M.S. (1973). Effect of age of the rat on induction of acetylcholinesterase in the brain by 17β-estradiol. Biochimica et Biophysica Acta, 329, 211-220.

Mueller, J.H., Rankin, J.L., and Carlomusto, M. (1979). Adult age differences in free recall as a function of basis of organization and method of presentation. Journal of Gerontology, 34, 375-380.

O'Keefe, J., and Nadel, L. (1978). The Hippocampus as a Cognitive Map. Oxford: Oxford University Press.

Oliverio, A., and Bovet, D. (1966). Effects of age on maze learning and avoidance conditioning of mice. Life Sciences, 5, 1317-1324.

Ordy, J.M., and Schjeide, O.A. (1973). Univariate and multivariate models for evaluating long-term changes in neurobiological development, maturity, and aging. In D.H. Ford (Ed.), Progress in Brain Research, Vol. 40: Neurobiological Aspects of Maturation and Aging, pp. 25-51. Amsterdam: Elsevier.

Parsons, P.J., and Spear, N.E. (1972). Long term retention of avoidance learning by immature and adult rats as a function of environmental enrichment. Journal of Comparative and Physiological Psychology, 80, 297-303.

Perlmutter, M. (1978). What is memory aging the aging of? Developmental Psychology, 154, 330-345.

Perlmutter, M. (1979). Age differences in adults' free recall, cued recall, and recognition. Journal of Gerontology, 34, 533-539.

Perry, E.K. (1980). The Cholinergic system in old age and Alzheimer's disease. Age and Ageing, 9, 1-8.

Perry, E.K., Perry, R.H., Gibson, P.H., Blessed, G., and Tomlinson, B.E. (1977). A cholinergic connection between normal aging and senile dementia in the human hippocampus, Neuroscience Letters, 6, 85-89.

Rankin, J.L., and Kausler, D.H. (1979). Adult age differences in false recognitions. Journal of Gerontology, 34, 58-65.

Reis, D.J., Ross, R.A., and Joh, T.H. (1977). Changes in the activity and amounts of enzymes synthesizing catecholamines and acetylcholine in brain, adrenal medulla, and sympathetic ganglia of aged rat and mouse. Brain Research, 136, 465-474.

Reisine, T.D., Yamamura, H.O., Bird, E.D., Spokes, E., and Enna, S.J. (1978). Pre- and postsynaptic neurochemical alterations in Alzheimer's disease. Brain Research, 159, 477-481.

Riege, W.H. (1971). Environmental influences on brain and behavior of year-old rats. Developmental Psychobiology, 4, 157-165.

Schonfield, D., and Robertson, B.A. (1966). Memory storage and aging. Canadian Journal of Psychology, 20, 228-236.

Sherman, K., Dallob, A., Dean, R.L., Bartus, R.T., and Friedman, E. (1980). Neurochemical and behavioral deficit in aging rats. Federation Proceedings, 39, 508.

Smith, A.D. (1975). Partial learning and recognition memory in the aged. International Journal of Aging and Human Development, 6, 359-365.

Strong, R., Hicks, P., Hsu, L., Bartus, R.T., and Enna, S.J. (1980). Age-related alterations in the rodent brain cholinergic system and behavior. Neurobiology of Aging, 1, 59-63.

Thompson, C.I., and Fitzsimons, T.R. (1976). Age differences in aversively motivated visual discrimination learning and retention in male Sprague-Dawley rats. Journal of Gerontology, 31, 47-52.

Timiras, P.S., and Vernadakis, A. (1972). Structural, biochemical, and functional aging of the nervous system. In P.S. Timiras (Ed.), Developmental Physiology and Aging, pp. 502-526. New York: Macmillan.

Tui, C., and Debruille, C. (1945). The comparative toxicity and effectiveness of scopolamine hydrobromide and scopolamine aminoxide hydrobromide. American Journal of Pharmacy, 117, 319-326.

Tulving, E., and Patkau, J.E. (1962). Concurrent effects of contextual constraint and word frequency of immediate recall and learning of verbal material. Canadian Journal of Psychology, 16, 83-95.

Vijayan, V.K. (1977). Cholinergic enzymes in the cerebellum and the hippocampus of the senescent mouse. Experimental Gerontology, 12, 7-11.

White, P., Hiley, C.R., Goodhardt, M.J., Carrasco, L.H., Keet, J.P., Williams, I.E.I., and Bowen, D.M. (1977). Neocortical cholinergic neurons in elderly people. Lancet, 1, 668-671.

Zelinski, E.M., Walsh, D.A., and Thompson, L.W. (1978). Orienting task effects on EDR and free recall in three age groups. Journal of Gerontology, 33, 239-245.

Zornetzer, S.F. (1978). Neurotransmitter modulation and memory: a new neuropharmacological phrenology? In M.A. Lipton, A. DiMascio, and K.F. Killam (Eds.), Psychopharmacology: A Generation of Progress, pp. 637-649. New York: Raven Press.

BIOGRAPHICAL SKETCH

Patricia Joan Kubanis was born in Bethesda, Maryland, on November 8, 1955, to Audrey S. and Ivan J. Kubanis. She grew up in San Diego, California and received a B.A. degree with Distinction in psychology and Highest Honors from San Diego State University in June, 1977. She was elected a member of Phi Beta Kappa and the recipient of a National Science Foundation Predoctoral Fellowship to attend the university of her choice. She was also awarded a fellowship by Phi Kappa Phi. She received the Doctor of Philosophy degree in the Department of Neuroscience in the College of Medicine at the University of Florida in August, 1981. She will enter the Ph.D. → M.D. Program in the University of Miami School of Medicine and plans to pursue a career in academic medicine.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Steven F. Zornetzer, Chairman
Associate Professor of Neuroscience

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Gerhard Freund
Professor of Medicine and Neuroscience

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Marieta B. Heaton
Associate Professor of Neuroscience

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James W. Simpkins
Assistant Professor of Pharmacy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Don W. Walker
Associate Professor of Neuroscience

This dissertation was submitted to the Graduate Faculty of the College of Medicine and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 1981

Dean, College of Medicine for 6/18/81

Madelyn Lockhart
Dean for Graduate Studies and Research